

11/28/00

Box SE9-A

12-04-00

11/28/00 U.S. PTO

DIVISION - CONTINUATION - CONTINUATION-IN-PART  
APPLICATION TRANSMITTAL FORM

Attorney Docket No.: A-579C

Anticipated Classification Of This Application:  
Class SubclassPrior Application:  
Examiner

Art Unit

To the Assistant Commissioner for Patents:

This is a request for filing a ☐ continuation ☐ divisional ☒ continuation-in-part application, under 37 CFR 1.53(b), of pending prior international application Serial No. PCT/US00/01871 filed on January 27, 2000, of Yoshinaga et al. for NOVEL POLYPEPTIDES INVOLVED IN IMMUNE RESPONSE

For CONTINUATION or DIVISIONAL APPLNs only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 1b, below, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

1. ☒ Transmitted herewith are:  
☒ 115 pages of specification, 7 pages of claim(s) and 1 page of abstract, totaling 123 pages.  
☒ 35 sheet(s) of drawings.  
☒ 4 pages of **Unsigned** Oath or Declaration by the applicant(s):  
☒ a. Newly executed (original or copy)  
☐ b. Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional applns. only)  
☒ 28 pages of Sequence Listing; computer readable format of sequence listing; sequence statement.
2. ☒ The filing fee is calculated below:

| For                       | Number Filed |        | Number Extra |   | Rate       | Fee        |
|---------------------------|--------------|--------|--------------|---|------------|------------|
| Total Claims              | 94           | - 20 = | 74           | x | \$18.00 =  | \$1,332.00 |
| Independent Claims        | 9            | - 3 =  | 6            | x | \$80.00 =  | 480.00     |
| Multiple Dependent Claims | 1            |        |              | + | \$270.00 = | 270.00     |
| Basic Fee                 |              |        |              |   | \$710.00 = | 710.00     |
| Total Filing Fee          |              |        |              |   |            | \$2,792.00 |

3. ☒ Please charge Deposit Account No. 01-0519, in the name of Amgen Inc., in the amount of \$2,792.00. An original and one copy are enclosed.
4. ☒ Throughout the prosecution of this application, if any extension of time is necessary, please consider this a request therefor.
5. ☒ The Commissioner is hereby authorized to charge any additional filing fees which may be required by the accompanying application, any additional fees which may be required during pendency of this application as required by 37 CFR 1.16 or 1.17, or credit any overpayment to Deposit Account No. 01-0519 throughout the prosecution of this application.
6. ☐ Cancel in this application original claims \_\_\_\_\_ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

## EXPRESS MAIL CERTIFICATE

"Express Mail" mail labeling number. EL360691010USDate of Deposit: November 28, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20531

Lynne Buchsbaum  
Printed NameLynne Buchsbaum  
Signature

7. ☒ Preliminarily, please amend the specification by inserting before the first line the following:

--This application is a ☒ continuation-in-part ☐ division of international application Serial No. PCT/US00/01871, filed January 27, 2000, which is a continuation-in-part of application Serial No. 09/264,527, filed March 8, 1999, which is a continuation of application Serial No. 09/244,448, filed February 3, 1999, which are hereby incorporated by reference.--

8. ☐ Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by § 1.138 and before payment of base issue fee.)

8a. ☐ New formal drawings are enclosed.

9. ☒ Priority of application Serial No. PCT/US00/01871 filed on January 27, 2000 in PCT (country) is claimed under 35 USC 119.

9a. ☐ The certified copy has been filed in prior application Serial No. \_\_\_\_\_ filed \_\_\_\_\_

10. ☒ The prior application is assigned of record to Amgen Inc.

11. ☐ A preliminary amendment is enclosed.

12. ☐ Also enclosed \_\_\_\_\_

13. ☐ Other: \_\_\_\_\_

14. ☒ The power of attorney in the prior application is to:  
Ron K. Levy, Registration No. 31,539; Steven M. Odre, Registration No. 29,094; and  
Robert B. Winter, Registration No. 34,458

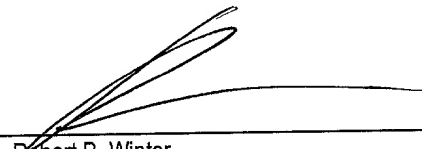
a. ☐ The power appears in the original papers in the prior application.

b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. ☒ Address all future communications to  
Robert B. Winter  
 at the address below.

Signator: ☐ Assignee of complete interest

☒ Attorney or agent of record

  
 Robert B. Winter  
 Attorney/Agent for Applicant(s)  
 Registration No. 34,458  
 Phone: (805) 447-2425  
 Date: November 28, 2000

Please send all future correspondence to:

U. S. Patent Operations/RBW  
 Dept. 4300, M/S 27-4-A  
 AMGEN, INC.  
 One Amgen Center Drive  
 Thousand Oaks, California 91320-1799

## NOVEL POLYPEPTIDES INVOLVED IN IMMUNE RESPONSE

This application is a continuation-in-part of international application Serial No. PCT/US00/01871, filed January 27, 2000, which is a continuation-in-part of application Serial No. 09/264,527, filed March 8, 1999, which is a continuation-in-part of application Serial No. 09/244,448, filed February 3, 1999, which are hereby incorporated by reference.

Field of the Invention

The present invention relates to polypeptides that are involved in T-lymphocyte activation. Specifically, the invention relates to T-lymphocyte costimulatory polypeptides, the nucleic acids encoding the polypeptides, expression vectors and host cells for production of the polypeptides, and compositions and methods for the treatment of diseases related to immunosuppression and immune activation.

Background of the invention

For the generation of a proper T-lymphocyte (T-cell) immune response, two signals must be provided to the T-cell by antigen presenting cells (APC). First, antigen must be presented to the T-cell receptor (TCR) via a major histocompatibility complex (MHC), in an event that determines specificity. Second, an antigen-independent, costimulatory signal must be delivered by engagement of members of the B7 family on the APC with the CD28 protein on T-cells. A productive immune response leads to proliferation, differentiation, clonal expansion, and effect or function. In the absence of the second, costimulatory signal, T-cells undergo a state of long-lasting antigen-specific unresponsiveness, termed anergy.

T-cells initiate the immune response, mediate antigen-specific effector functions, and regulate the activity of other leukocytes by secreting cytokines. The T-cell receptor (TCR) distinguishes the T-cell from other lymphocytes and can bind antigen only when it is presented by the APC within the context of a MHC. The functional activity of a particular T-cell can be correlated with the expression of membrane antigens, such as CD4 and CD8. For instance, CD4+ T-cells generally function as T helper cells ( $T_H$ ) and are MHC class II restricted, whereas CD8+ cells generally function as cytotoxic T-cells ( $T_C$ ) and are MHC class I restricted.

Potent T-cell costimulatory polypeptides which has been previously identified include polypeptides termed B7.1 (Freeman et al. J. Immunology 143, 2714-2722 (1989), Freeman et al. Jour. Expt. Med. 174, 625-31 (1991)) and B7.2 (Freeman et al. Science 262, 909-911 (1993), and Freeman et al. Jour. Expt. Med. 178, 2185-2192 (1993)), (or CD80 and CD86, respectively). These polypeptides are either inducibly or constitutively expressed on various APCs and are membrane-bound ligands for CD28 and CTLA-4, respectively, on T-cells. CD28 (Aruffo and Seed Proc. Natl. Acad. Sci. 84, 8573-8577 (1987) and Gross et al. J. Immun. 144, 3201-3210 (1990)) is expressed on resting T-cells and mediates a positive costimulatory signal. CTLA-4 (Brunet et al. Nature 328, 267-270 (1987) and Dariavach et al. Eur. Jour. Immun. 18, 1901-1905 (1988)) expression is induced upon T-cell activation and negatively regulates the CD28 signal, due to its higher binding affinity for B7.1 and B7.2. Mice without the CTLA-4 gene exhibit dramatically high levels of T-cells, since the switch off mechanism for the proliferation signal is impaired in the absence of



5

10

20

30

## Summary of the Invention

35

unique costimulatory pathway which appears to be distinct from the pathway consisting of previously described proteins CD28, CTLA-4, B7.1, and B7.2. The polypeptides are referred to as CD28 related protein-1, or CRP1, and B7 related protein-1, or B7RP1.

The invention provides for nucleic acid molecules encoding CRP1 and B7RP1 polypeptides and related polypeptides. An isolated nucleic acid molecule of the invention comprises a nucleotide sequence selected from the group consisting of:

- a) the nucleotide sequence as set forth in Figure 1A (SEQ ID NO:1);
- b) the nucleotide sequence encoding the polypeptide from residues 1-200 or from residues 21-200 as set forth in Figure 1A (SEQ ID NO:1);
- c) a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in Figure 1A (SEQ ID NO: 1);
- d) a naturally occurring allelic variant or alternate splice variant of any of (a), (b) or (c );
- e) a nucleotide sequence complementary to any of (a), (b) or (c );
- f) a nucleotide sequence of (b), (c ) or (d) encoding a polypeptide fragment of at least about 25, 50, 75, 100, or greater than 100 amino acid residues;
- g) a nucleotide sequence of (a), (b) or (c ) comprising a fragment of at least about 10, 15, 20, 25, 50, 75, 100, or greater than 100 nucleotides; and
- h) a nucleotide sequence which hybridizes under stringent conditions to any of (a)-(g).

Also provided by the invention is an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence as set forth in Figure 2A (SEQ ID NO: 6) or Figure 3A (SEQ ID NO:11) or Figure 12A (SEQ ID NO: 16);

b) the nucleotide sequence encoding the polypeptide as set forth in Figure 2A (SEQ ID NO: 6) from residues 1-322 or from residues 47-322 or as set forth in Figure 3A (SEQ ID NO:11) from residues 1-288 or from residues 19-288, 20-288, 21-288, 22-288, 24-288, or 28-288; or as set forth in Figure 12A from residues 1-302 or from residues 19-302, 20-302, 21-302, 22-302, 24-302 or 28-302;

c) a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in Figure 2A (SEQ ID NO: 6) or Figure 3A (SEQ ID NO: 11) or Figure 12A (SEQ ID NO: 16);

d) a naturally occurring allelic variant or alternate splice variant of any of (a), (b) or (c );

e) a nucleotide sequence complementary to any of (a), (b) or (c );

f) a nucleotide sequence of (b), (c ) or (d) encoding a polypeptide fragment of at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

g) a nucleotide sequence of (a), (b) or (c ) comprising a fragment of at least about 10, 15, 20, 25, 50, 75, 100, or greater than 100 nucleotides; and

h) a nucleotide sequence which hybridizes under stringent conditions to any of (a)-(g).

The subject matter of the invention also relates to CRP1 and B7RP1 polypeptides and related polypeptides. The invention provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

a) the amino acid sequence as set forth in Figure 1A (SEQ ID NO:2);

b) the mature amino acid sequence as set forth in Figure 1A (SEQ ID NO:2) comprising a mature amino terminus at residue 21;

c) a fragment of the amino acid sequence set forth in Figure 1A (SEQ ID NO: 2) comprising at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

d) an ortholog of (a), (b) or (c ); and

e) an allelic variant or alternative splice  
10 variant of (a), (b) or (d).

Also in accordance with the invention is an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

15           a) the amino acid sequence as set forth in  
Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID NO:12) or  
Figure 12A (SEQ ID NO: 17);

b) the mature amino acid sequence as set forth in Figure 2A (SEQ ID NO: 7) comprising a mature amino terminus at residues 47 or Figure 3A (SEQ ID NO:12) comprising a mature amino terminus at any of residues 19, 20, 21, 22, 24 or 28 or Figure 12A (SEQ ID NO: 17) comprising a mature amino terminus at any of residues 19, 20, 21, 22, 24, or 28;

25                   c) a fragment of the amino acid sequence set forth in Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID NO: 12) or Figure 12A (SEQ ID NO: 17) comprising at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

30                   d) an ortholog of (a), (b) or (c ); and  
                    e) an allelic variant or alternative splice  
variant of (a), (b), (c) or (d).

Also encompassed by the invention are  
35 expression vectors and host cells for production of the  
polypeptides, antibodies which bind to CRP1 and B7RP1

polypeptides and to related polypeptides, and assays for detecting binding of B7RP1 and B7RP1-related polypeptides to CRP1 and CRP1-related polypeptides.

Pharmaceutical compositions comprising CRP1 or

5 CRP1-related polypeptides and B7RP1 or B7RP1-related polypeptides are also encompassed by the invention. Methods for identifying compounds that interact with CRP1 or B7RP1 are also provided as are assays for determining whether such compounds are agonists or  
10 antagonists of CRP1 and B7RP1 activity.

CRP1 and B7RP1 polypeptides are involved in T-cell costimulation and proliferation. CRP1 and B7RP1 polypeptides, selective binding agents thereof, and agonists and antagonists thereof, may be useful for the diagnosis, prevention and treatment of diseases related to the control of T-cell responses.

CRP1 and B7RP1 polypeptides, selective binding agents thereof, and agonists and antagonists thereof, may be useful for the diagnosis, prevention and treatment of immune disorders, either for stimulating insufficient immune response or reducing or inhibiting an exaggerated or inappropriate immune response. The immune disorder may be mediated directly or indirectly by T-cells.

25           The invention provides for treating,  
preventing, or ameliorating a T-cell mediated disorder  
comprising administering to an animal a CRP1 or B7RP1  
polypeptide. The invention also provides for a method  
of diagnosing a T-cell mediated disorder or a  
30   susceptibility to a T-cell mediated disorder in an  
animal comprising determining the presence or amount of  
expression of a CRP1 or B7RP1 polypeptide; and  
diagnosing a T-cell mediated disorder or a  
susceptibility to a T-cell mediated disorder based on  
35   the presence or amount of expression of the  
polypeptide. Typically, a T-cell mediated disorder is

an immune disorder which may be mediated directly or indirectly by T-cells. The animal is preferably a mammal and more preferably a human. The invention also provides for a method of identifying a test molecule  
5 which binds to a CRP1 or B7RP1 polypeptide comprising contacting the polypeptide with a test compound and determining the extend of binding of the polypeptide to the test compound. The method may be used to identify agonists and antagonists of CRP1 and/or B7RP1  
10 polypeptide.

Antagonists of CRP1 and/or B7RP1 polypeptides may be used as immunosuppressive agents for many indications, including autoimmune disorders (such as rheumatoid arthritis, psoriasis, multiple sclerosis,  
15 diabetes, and systemic lupus erythematosus), toxic shock syndrome, bone marrow and organ transplantation, inflammatory bowel disease, allosensitization due to blood transfusions, and the treatment of graft vs. host disease. In addition, antagonists may be used as  
20 inhibitory agents for T-cell dependent B-cell mediated indications including asthma and allergy, and antibody mediated autoimmunity. Agonists of the CRP1 and/or B7RP1 polypeptides may be useful in, but not restricted to, T-cell activation for tumor surveillance and  
25 removal.

An antagonist of the invention includes an antibody, or fragment thereof, which is reactive with or binds to B7RP1 or to an extracellular domain of B7RP1 wherein the antibody reduces or eliminates the  
30 binding to B7RP1 to CRP1. In one embodiment, the antibody binds selectively to human B7RP1 or to an extracellular domain thereof. The antibody or fragment thereof which is an antagonist inhibits partially or completely the immune costimulatory activity of B7RP1.  
35 In a preferred embodiment, the antibody is a monoclonal

antibody and may be murine, human, chimeric or humanized.

The invention further provides for a method of regulating the interaction of B7RP1 with CRP1 comprising administering to an animal a selective binding agent of CRP1 or a selective binding agent of B7RP1 or both. In one embodiment, the selective binding agent is an antibody which binds to B7RP1 and reduces or eliminates the binding to B7RP1 to CRP1. The invention also provides a method of regulating immune costimulation mediated by B7RP1 comprising administering to an animal a selective binding agent of B7RP1. The selective binding agent is preferably an antibody which binds to B7RP1 and partially or completely inhibits immune costimulation mediated by B7RP1.

The invention also provides for a method of regulating T-cell activation or proliferation in an animal comprising administering to the animal a nucleic acid molecule encoding a CRP1 or B7RP1 polypeptide. For example, a nucleic acid molecule encoding a B7RP1 polypeptide may be used in gene therapy to enhance T-cell activation in response to various tumors.

Also encompassed by the invention is a transgenic non-human mammal comprising a nucleic acid molecule encoding a CRP1 or B7RP1 polypeptide. The CRP1 or B7RP1 nucleic acids are introduced into the mammal in a manner that allows expression and increased circulation levels of CRP1 or B7RP1 polypeptides. The transgenic non-human mammal is preferably a rodent, and more preferably a mouse or a rat.

A method for stimulating or enhancing an immune response comprising administering B7RP1 or CRP1, or a B7RP1 agonist or CRP1 agonist is also provided. Optionally, an immune response may be stimulated or enhanced by further administering one or more other

immune stimulating molecules, such as a CD28 agonist, a CTLA4 antagonist, or molecules such as B7.1 and/or B7.2.

Also provided by the invention is a method of regulating IgE production comprising administering B7RP1 or CRP1, or a combination thereof, a B7RP1 agonist or a CRP1 agonist, or a combination thereof, or a B7RP1 antagonist or a CRP1 antagonist, or a combination thereof. For example, administration of B7RP1 or a combination of B7RP1 and a CRP1 agonist would increase IgE production and would be useful for stimulating an insufficient IgE-mediated immune response. Administration of a B7RP1 antagonist or a CRP1 antagonist, or a combination thereof, would decrease IgE production and would be useful for inhibiting an exaggerated or inappropriate IgE-mediated immune response. In one embodiment, IgE production is partially or completely inhibited by administration of a B7RP1 antagonist, or a CRP1 antagonist, or a combination thereof. In another embodiment, the invention provides for a method of preventing and/or treating an IgE-mediated disorder comprising administering a B7RP1 antagonist, or a CRP1 antagonist, or a combination thereof. IgE-mediated disorders include those characterized by excessive IgE production, such as asthma, allergic disorders, hypersensitivity and sinus inflammation.

#### Description of Figures

Figure 1. A) DNA and amino acid sequence murine CRP1 (mCRP1). Predicted signal sequence of CRP1 is underlined at the amino-terminus and the experimentally determined pro-peptide cleavage site is indicated by an asterisk. Predicted transmembrane sequence is underlined toward the carboxy-terminus. B)



Amino acid alignment of murine CRP1 protein sequence (mCRP1) with murine CD28 (mCD28).

Figure 2. A) DNA and amino acid sequence of murine B7RP1 (mB7RP1). Predicted signal sequence of B7RP1 is underlined at the amino-terminus and the experimentally determined pro-peptide cleavage site is indicated by an asterisk. Predicted transmembrane sequence is underlined toward the carboxy-terminus. B) Amino acid alignment of B7RP1 protein sequence (mB7RP1) with murine CD80 (mCD80).

Figure 3. A) Structure and sequence of the protein coding region of the putative human B7RP1 (hB7RP1). Predicted signal sequence of hB7RP1 is underlined at the amino-terminus. Predicted signal peptide cleavage sites are marked by asterisks. Predicted transmembrane sequence is underlined toward the carboxy-terminus. B) Amino acid alignment of the putative mature hB7RP1 protein with the mature murine B7RP1 (mB7RP1) protein.

Figure 4. A) Expression of soluble CRP1-Fc fusion protein from 293T-cells transfected with the pcDNA3/CRP1-Fc. Normalized volumes of cell lysate or conditioned medium were loaded and separated on a 10% PAGE gel as indicated. Western analysis of cell lysate and cell media supernatant for expression of cell-associated (cell lysate) and secreted (media) Fc fusion proteins. Primary antibody was Goat-anti human Fc antibody (Pierce Chemical Company, Rockford, IL.). B) Expression of soluble B7RP1-Fc fusion protein from 293T-cells transfected with the pcDNA3/ B7RP1-Fc. 20  $\mu$ l of normalized cell lysate or media supernatant were loaded and separated on a 10% PAGE gel. Western analysis was conducted as in (A).

Figure 5. Interaction of CRP1-Fc and B7RP1-Fc fusion proteins with membrane-bound proteins expressed in COS-7 cells. COS-7 cells transiently transfected with pcDNA3/CRP1, pcDNA3/B7RP1, or pcDNA3 vector alone. CHO D-cells were transfected with psDR $\alpha$ /hCD28 and stably expressed human CD28 (hCD28). Cells expressing membrane-bound CRP1, B7RP1, or hCD28, are represented in rows as indicated at the left side of the panel. Fc fusion proteins were incubated with the plate-bound cells in columns as indicated at the top of the panel. After incubation, cells were washed, and bound Fc fusion proteins were detected using an anti-human Fc antibody and ACAS (Adherent Cell Analysis and Sorting; ACAS Ultima, Meridian Instruments, Inc., Okemos, MI) analysis.

Figure 6. FACS (Fluorescence-Activated Cell Sorter) analysis of expression of the receptor for B7RP1 (putatively, CRP1) on activated CD4+ and CD8+ T-cells. Mouse splenocytes were activated with PMA and ionomycin for 12 hours. B7RP1-Fc fusion protein, control Fc protein (Mock-Fc), or PBS (no stain), were incubated with the cells, washed, and subsequently incubated with goat-anti-human Fc-FITC conjugated antibody (GaHuFc-FITC) as indicated at the bottom of each panel. Cell marker antibodies (for T-cell markers CD4 and CD8) PE conjugated, or isotype control antibody (rat isotype) PE conjugated, or PBS (no stain), were added as indicated at the left side of each individual panel.

Figure 7. FACS (Fluorescence-Activated Cell Sorter) analysis of the expression of B7RP1 on B-cells. Fluorocytometric analysis of the expression of the

ligand for CRP1 (presumably, B7RP1) on mouse splenocytes. CRP1-Fc fusion protein, control Fc protein (Mock-Fc), or PBS (no stain), were incubated with the cells, washed, and subsequently incubated with goat-anti-human Fc-FITC conjugated antibody (GaHuFc-FITC) as indicated at the bottom of each panel. PE conjugated cell marker antibody to CD45R (CD45R is a B-cell marker) or isotype control antibody (rat isotype), or PBS (no stain), were added as indicated at the left side of each individual panel.

Figure 8. FACS analysis of the expression of mCRP1 ligand on peritoneal macrophages. Peritoneal cells were first distinguished in subsets on the ground of their light scattering properties (panel A). Macrophages were identified in region 5 (R5) because of their ability to strongly scatter light forward (FSC) and sideways (SSC) and because of their positive staining for the F4/80 antigen, a marker for macrophages (panel B). Macrophages in region 6 (R6) were singled out on the basis of their less intense staining for the F4/80 antigen and found to be stained by the CRP1-Fc fusion protein (presumably because of their expression of B7RP1).

Figure 9. Inhibition of T-cell proliferation using a B7RP1-Fc fusion protein. T-cells from mouse splenocytes were activated by increasing concentrations of Concanavalin A (Con A) as indicated at the bottom of the graph. mCRP1-Fc, mB7RP1, and mB7.2-Fc fusion proteins were added to enriched T-cells from splenocytes in the absence (no adds) or presence of Con A. 200,000 cells were used in the T-cell proliferation assays in a 96-well plate. Cells were incubated with media (no adds) or Fc fusion proteins as indicated in the graph legend. After 42 hr, cells were pulsed with

H-thymidine for 6 hr, then harvested and incorporated radioactivity determined. Average CPM and standard deviation from triplicate samples are represented.

- 5                    Figure 10. A) Normal mesenteric lymph node from control Mouse #10 showing the cortex, paracortex and medulla of the node. Hematoxylin-eosin (H&E) stain, 40x magnification. B) Markedly enlarged mesenteric lymph node from WX11 Mouse #40 with prominent
- 10 follicular hyperplasia (FH), expansion of paracortex and medullary cord hyperplasia (MH). H&E, 40x. C) Close-up of the medullary cords (MC) and sinuses (MS) from the mesenteric lymph node of control Mouse #10. Note the small medullary cords composed of mostly small
- 15 lymphocytes adjacent to medullary sinuses with fleshy macrophages. H&E, 400x. D. Close-up of the medullary cords (MC) and sinuses (MS) from the mesenteric lymph node of WX11 Mouse #40. Note the markedly thickened medullary cords composed of large numbers of plasma
- 20 cells with occasional Russell body cells (arrow). H&E, 400x. E) Normal spleen from control Mouse #10 showing red pulp and white pulp areas with periarteriolar lymphoid sheaths (PALS), 100x. *Inset*: close-up of the marginal zone surrounding the white pulp with small
- 25 lymphocytes, macrophages and occasional plasma cells, 400x. F) Spleen from WX11 Mouse #6 with enlarged white pulp areas, including PALS and follicles (arrow), 100x. *Inset*: close-up of the marginal zone with numerous plasma cells and occasional Russell bodies, 400x. G)
- 30 Ileum with Peyer's patch from control Mouse #25 with the interfollicular zone (arrow) flanked by two secondary follicles, 40x. H) Ileum with Peyer's patch from WX11 Mouse#32 with markedly enlarged follicles with prominent germinal centers and interfollicular
- 35 tissue (arrow), 40x.

Figure 11. A) Normal mesenteric lymph node from control Mouse #5 showing the cortex, paracortex and medulla of the node. Hematoxylin-eosin (H&E) stain, 40x magnification. B) Markedly enlarged mesenteric lymph node from WX11 Mouse #33 with prominent follicular hyperplasia (*top*: rows of secondary follicles in the outer cortex), expansion of the paracortex (*center*) and medullary cord hyperplasia (*bottom*). H&E, 40x. C) Immunohistochemical staining of the mesenteric lymph node from control Mouse #10 with anti-B220 antibody (B cell marker). Note the intensely (brown) staining cortical area and thin medullary cords. Immunostaining performed using the avidin-biotin complex (ABC) immunoperoxidase method (DAB chromogen, hematoxylin counterstain), 40x. D) Immunohistochemical staining of the mesenteric lymph node from WX11 Mouse #33 with anti-B220 antibody. Note the intensely staining cortical follicles and medullary cords (although the mature plasma cells in the cords are negative for B220), 40x. E) Immunohistochemical staining of the lymph node from control Mouse #10 with anti-CD3 antibody (T-cell marker). Note the immunostaining of the paracortical zone of the node, 40x. F) Immunohistochemical staining of the lymph node from WX11 Mouse #33 with anti-CD3 antibody. Note the enlarged, intensely staining paracortical areas of the node, 40x.

Figure 12. A) Structure and sequence of the protein coding region of human B7RP1 (hB7RP1). Predicted signal sequence of hB7RP1 is underlined at the amino-terminus. Predicted signal peptide cleavage sites are marked by asterisks. Predicted transmembrane sequence is underlined toward the carboxy-terminus. B) Amino acid alignment of the putative mature hB7RP1 protein with the mature murine B7RP1 (mB7RP1) protein.

Figure 13. A) Structure and sequence of the protein coding region of human CRP1 (hCRP1). Predicted signal sequence of hCRP1 is underlined at the amino-terminus. Predicted signal peptide cleavage sites are marked by asterisks. Predicted transmembrane sequence is underlined toward the carboxy-terminus. B) Amino acid alignment of the hCRP1 protein with the murine CRP1 (mB7RP1) protein.

Figure 14. CRP1 is on resting memory T-cells. Resting splenocytes from 6-7 month old mice were double-stained using B7RP1-Fc labeled by an FITC-conjugated anti-human Fc antibody and a PE-conjugated antibody to either CD44(Fig 14A), CD45RB(Fig 14B), or CD69(Fig 14C).

Figure 15. T-cell co-stimulation by B7RP1-Fc fusion protein. A) T-cell proliferation induced by different quantities of B7RP1-Fc (closed squares), B7.2-Fc (closed circles), or OPG-Fc fusion protein control (open squares) in conjunction with anti-CD3 antibody. Fusion proteins were used at various concentrations to coat 96 well plates pre-coated with anti-human Fc FAb<sub>2</sub> (12.5 µg/ml) and anti-CD3 antibody (0.9 µg/ml). B7RP1-Fc and B7.2-Fc co-stimulate T-cells in a dose-dependent fashion up to 0.3 µg/ml, at which the maximal effect is achieved. B) T-cell proliferation induced by B7RP1-Fc (closed squares), B7.2-Fc (closed circles), non-fused Fc (open squares), or no Fc (open circles) in conjunction an anti-CD3 antibody (0.85 µg/ml) and in the presence of various concentrations of a rabbit anti-B7RP1-Fc polyclonal antibody. Fc fusion proteins were used at a concentration of 0.3 µg/ml and were bound to the plates as above. The anti-B7RP1-Fc antibody was raised to

purified B7RP1-Fc by subcutaneous injections of antigen emulsified in adjuvant, and then was affinity purified. The antibodies were incubated for 30 min with the Fc fusion proteins before the addition of the cells. The anti-B7RP1-Fc antibody specifically inhibits the T-cell proliferation induced by B7RP1-Fc in a dose-dependent fashion.

Figure 16. Effect of CRP1-Fc and B7RP1-Fc proteins on the incidence (A) and severity (B) of collagen induced arthritis in mice. Collagen induced arthritis susceptible B10.RIII mice were immunized at the base of the tail with 10 µg porcine collagen type II in CFA. Mice received 100 µg of fusion protein twice per week. Fc fusion proteins and control PBS treatment are indicated in the figure legend.

Figure 17. Proximal Colon in B7RP1-Fc Transgenic Mice. (A) Normal proximal colon from control Mouse#53F (female) showing the gut wall with mucosa, submucosa, muscularis and serosa. Hematoxylin-eosin (H&E) stain, 40x magnification. (B) Diffusely thickened proximal colon from B7RP1-Fc transgenic Mouse#111F with prominent glandular hypertrophy, fissuring ulceration and transmural inflammation. H&E, 40x. (C) Lower power view of proximal colon (as in panel B) from B7RP1-Fc transgenic Mouse#111F with multifocal fissuring ulceration and transmural inflammation. H&E, 20x. (D) Close-up of the fissuring ulcer and hypertrophic colonic glands from B7RP1-Fc transgenic Mouse#111F (shown in panels B and C above). Note the lumen with mucopurulent exudate. H&E, 100x. (E) Close-up of granulomatous inflammation in the submucosa of B7RP1-Fc transgenic Mouse#112F with a multinucleated giant cell surrounded by macrophages,

5

25

35



B7RP1-Fc transgenic Mouse#51F (female) with prominent crypt hypertrophy and hyperplasia and mild lymphoplasmacytic infiltrate in the lamina propria.

H&E, 40x. (C) Normal jejunum from control mouse #53F

5 (female) showing the normal length of the villi and crypts in the jejunal mucosa. H&E, 40x magnification.

(D) Markedly thickened jejunal mucosa from B7RP1-Fc transgenic Mouse#51F (female) with locally extensive crypt hypertrophy and hyperplasia. H&E, 40x. (E)

10 Normal ileum from control Mouse#53F (female) showing the normal length of the villi and crypts in the ileal mucosa. H&E, 40x magnification. (F) Mild atrophy of ileal mucosa from B7RP1-Fc transgenic Mouse#231M (male) with focal loss and blunting of villi. H&E, 40x.

15

Figure 20. The B7RP1-Fc fusion protein inhibits tumor growth in mice. Meth A sarcoma cells were implanted intradermally in the abdomen of Balb/C mice. On days 7, 10, 14, and 17, after implantation,

20 the mice were treated with vehicle (dark diamonds) or murine B7RP1-Fc (gray triangles, Example 7). Tumor volume was measured, as described in Example 20, on the indicated days after implantation. The tumor growth was monitored up to day 28. Each group had eight mice.

25

Figure 21. T-cell co-stimulation by human B7RP1-Fc. Anti-CD3 and human B7RP1 Fc were used to coat 96 well plates, and  $1 \times 10^5$  T-cells/well (>98% CD3+) were cultured and harvested as described in

30 Example 21. A) Co-stimulation induced by anti-CD3 only (closed circles), 0.5  $\mu\text{g/ml}$  B7RP1 Fc (closed triangles), 0.5  $\mu\text{g/ml}$  OPG-Fc (open circles), and 5  $\mu\text{g/ml}$  anti-CD28 (open triangles) at different concentrations of anti-CD3 primary stimulation. Data

35 show that B7RP1-Fc co-stimulated anti-CD3 primed T-

cells to similar levels as co-stimulation using anti-CD28 antibodies. Data shown are mean [<sup>3</sup>H]TdR incorporated +/- SD in triplicate wells from one representative experiment of several experiments generated with T-cells isolated from three normal donors. B) Dose-dependent inhibition of B7RP1-Fc co-stimulation by CRP1-Fc. T-cells were cultured in wells coated with both anti-CD3 at 0.3 µg/ml and 0.5 µg/ml B7RP1-Fc. Serially diluted concentrations of CRP1-Fc (closed circles) or OPG-Fc (open circles) were preincubated with the B7RP1-Fc for 30 min prior to the addition of T-cells. Data show that CRP1-Fc inhibits B7RP1 induced co-stimulation in a dose-dependent manner. Percent inhibition is plotted against CRP1-Fc or OPG-Fc protein concentration. Data shown are mean [<sup>3</sup>H]TdR incorporated +/- SD of three experiments done in triplicate wells and are representative of experiments generated with two normal donors. C) Co-stimulation by CHO human B7RP1 cells. T-cells were purified from peripheral blood and were cultured with various concentrations of anti-CD3 in the presence of anti-CD3 alone (closed circles), 1 x 10<sup>4</sup> CHO vector control cells (open circles) or 1 x 10<sup>4</sup> CHO B7RP1 cells (closed triangles), as described in Example 22. The data show that membrane-bound B7RP1 co-stimulated T-cell growth to a level similar to that observed using B7RP1-Fc fusion proteins. Data shown are the mean +/- SD of triplicate cultures and are representative of results generated with two normal donors. D) Cytokine production. T-cells were cultured as described in (Figure 21A) and supernatants were collected at 48 (black bars) and 72 (gray bar) hrs. Data show that the amount of IL-2 produced by B7RP1-Fc co-stimulated cells (top graph) was similar to that produced by cells stimulated by anti-CD3 and control Fc,

but significantly less than that produced by anti-CD28 co-stimulated cells. Data also show that B7RP1-Fc co-stimulation enhanced IL-10 (middle graph) and IFN-gamma (bottom graph) production.

5

Figure 22 shows IgE levels in control and B7RP1 transgenic mice.

Figure 23 shows the combined effects of B7RP1-Fc and B7.2-Fc on chronic hypersensitivity. Mice were challenged with oxazolone as described in Example 24. B7RP1-Fc, B7.2-Fc, B7RP1-Fc+B7.2-Fc, and Fc were given at high (2 or 1+1 mg/Kg, A) and low (0.4 or 0.2+0.2 mg/Kg, B) dose around the time of challenge. A, Compared to Fc, B7RP1-Fc B7.2-Fc, and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET from day 3 ( $p < 0.001$ ). B7RP1-Fc and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET more than B7.2-Fc from day 4 ( $p < 0.001$ ). B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET more than B7RP1-Fc from day 4 ( $p < 0.001$ ). B, Compared to Fc, B7RP1-Fc and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET from day 4 ( $p < 0.001$ ). B7RP1-Fc and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET more than B7.2-Fc ( $p < 0.001$ ).

#### Detailed Description of the Invention

The invention provides for novel polypeptides referred to herein as CRP1 and B7RP1, which comprise a receptor-ligand pair that is involved in T-cell activation. cDNAs encoding the polypeptides were identified from a library prepared from mouse intestinal intraepithelial cells and screened on the basis of homology to the CD28 and CTLA-4 polypeptides (for CRP1) or B7.1 and B7.2 polypeptides (for B7RP1).

CD28 related protein-1, or CRP1, is predicted to be a type I transmembrane protein with a signal

sequence and extracellular domain at the amino-terminus, a transmembrane domain, and a carboxy terminal intracellular domain (Figure 1). The full-length CRP1 protein is 180 amino acids in its mature  
5 form. The predicted leader sequence spans about amino acid residues 1-20 (relative to the initiating methionine) and the extracellular domain of the mature protein encompasses about residues 21-145 (Example 1). The predicted transmembrane domain spans about residues  
10 146-163 and the intracellular domain encompasses about residues 164-200. The amino terminal extracellular domain is similar to an Ig loop with conserved putative intra- and inter-molecular bonding cysteines. Furthermore, a "MYPPPY" motif, which is previously  
15 known to be important for B7.1 and B7.2 binding to CD28 and CTLA-4, is also partially conserved.

CD28 and CTLA-4 are weakly homologous as exemplified by the 26% amino acid identity between murine CD28 and CTLA-4. There is 19% amino acid  
20 identity of CRP1 with murine CD28 and 14% identity of CRP1 with murine CTLA-4. However, critical cysteine residues are conserved between murine CD28, CTLA-4 and CRP1 at residues 42, 63, 83, 109, and 137 (relative to the initiating methionine in the CRP1 protein, See  
25 Figure 1A). The approximate mature protein lengths and locations of the transmembrane region relative to the carboxy terminus are also similar in CRP1, CD28, and CTLA-4.

Human CRP1 is a transmembrane protein having  
30 the nucleotide and amino acid sequence as shown in Figure 13A. The predicted leader sequence spans about residues 1-19 or about residues 1-20. The predicted mature amino terminus is at residues 20 or 21. Preferably, the mature amino terminus is at position  
35 21. The extracellular domain spans from any of the predicted mature amino termini to about amino acid

B7 related protein-1, or B7RP1, is predicted to be a type I transmembrane protein with a signal sequence and extracellular domain at the

B7.1 and B7.2 are weakly homologous as exemplified by the 24% amino acid identity between murine B7.1 and B7.2. There is 20% amino acid identity of B7RP1 with murine B7.1 and 19% identity of B7RP1 with murine B7.2. However, critical cysteine residues are conserved between murine B7.1, B7.2 and B7RP1 at residues 62, 138, 185, and 242 (relative to the initiating methionine in the B7RP1 protein, Figure 2A). The approximate mature protein length and location of the transmembrane region relative to the carboxy terminus are also similar in mB7RP1, B7.1, and B7.2.

Human B7RP1 is also a transmembrane protein with conserved cysteine residue in the extracellular domain which are necessary for Ig loop structures. The predicted leader sequence encompasses about residues 1-

extracellular domain spans from any of the mature amino termini to about amino acid residue 259. The predicted transmembrane domain spans about residues 259-274. The intracellular domain encompasses residues 275-302. The full-length human B7RP1 nucleotide and amino acid sequence is shown in Figure 12A. Human B7RP1 is about 43% identical to the murine protein.

CRP1 and B7RP1 bind each other, but CRP1 does not detectably bind to the B7RP1 related protein B7.2; and B7RP1 does not exhibit detectable binding to CRP1-related CD28 or CTLA-4 (Example 8). B7RP1 was shown to regulate T-cell proliferation, presumably through the interaction of B7RP1 with CRP1 receptors (Example 11). Thus, CRP1 and B7RP1 represent a novel pathway for regulating T-cell proliferation and activation.

The interaction of B7RP1 with CRP1 can be regulated in such a manner that immune costimulation and T-cell proliferation and activation can be increased or decreased. By way of example, anti-B7RP1 monoclonal and polyclonal antibodies raised against murine B7RP1 blocked the B7RP1/CRP1 interaction and also blocked T-cell proliferation induced by a B7RP1-Fc fusion protein (see Example 17). A human CRP1-Fc fusion protein blocked human T-cell proliferation induced by human B7RP1-Fc (Example 21). In addition, addition of a CRP1-Fc fusion protein delayed the onset of arthritic symptoms in a mouse model of rheumatoid arthritis (see Example 18). B7RP1/CRP1 co-stimulation can also be increased by addition of B7RP1-Fc fusion protein or other activators of this pathway (Example 20).

| Table 1. Demographic characteristics of the study population |              |
|--|--------------|
| <b>Age (years)</b>   |              |
| 18-24  | 10 (10.0%)   |
| 25-34  | 15 (15.0%)   |
| 35-44  | 20 (20.0%)   |
| 45-54  | 25 (25.0%)   |
| 55-64  | 30 (30.0%)   |
| 65-74  | 35 (35.0%)   |
| 75-84  | 40 (40.0%)   |
| 85-94  | 45 (45.0%)   |
| 95-104   | 50 (50.0%)   |
| 105-114  | 55 (55.0%)   |
| 115-124  | 60 (60.0%)   |
| 125-134  | 65 (65.0%)   |
| 135-144  | 70 (70.0%)   |
| 145-154  | 75 (75.0%)   |
| 155-164  | 80 (80.0%)   |
| 165-174  | 85 (85.0%)   |
| 175-184  | 90 (90.0%)   |
| 185-194  | 95 (95.0%)   |
| 195-204  | 100 (100.0%) |
| <b>Gender</b>  |              |
| Male   | 50 (50.0%)   |
| Female   | 50 (50.0%)   |
| <b>Ethnicity</b>   |              |
| White  | 30 (30.0%)   |
| Black  | 20 (20.0%)   |
| Hispanic   | 10 (10.0%)   |
| Asian  | 10 (10.0%)   |
| Other  | 20 (20.0%)   |
| <b>Education</b>   |              |
| High school or less  | 20 (20.0%)   |
| Some college   | 20 (20.0%)   |
| Bachelor's degree  | 20 (20.0%)   |
| Master's degree  | 10 (10.0%)   |
| PhD  | 10 (10.0%)   |
| <b>Income</b>  |              |
| <\$10,000  | 10 (10.0%)   |
| \$10,000-\$19,999  | 20 (20.0%)   |
| \$20,000-\$29,999  | 20 (20.0%)   |
| \$30,000-\$39,999  | 20 (20.0%)   |
| \$40,000-\$49,999  | 20 (20.0%)   |
| \$50,000-\$59,999  | 20 (20.0%)   |
| \$60,000-\$69,999  | 20 (20.0%)   |
| \$70,000-\$79,999  | 20 (20.0%)   |
| \$80,000-\$89,999  | 20 (20.0%)   |
| \$90,000-\$99,999  | 20 (20.0%)   |
| \$100,000+   | 20 (20.0%)   |
| <b>Health status</b>   |              |
| Good   | 30 (30.0%)   |
| Fair   | 20 (20.0%)   |
| Poor   | 10 (10.0%)   |
| Very poor  | 10 (10.0%)   |
| Unknown  | 20 (20.0%)   |

10           The term "allelic variant" refers to one of  
several possible naturally occurring alternate forms of  
a gene occupying a given locus on a chromosome of an  
organism.

The term "high stringency conditions" refers to those conditions which: (1) employ low ionic strength and high temperature for washing, for example, 0.1 X SSC (0.015 M NaCl/0.0015 M sodium citrate) 0.1% NaDodSO<sub>4</sub> (SDS) at 50°C, or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1%. Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 5 X SSC (750 mM NaCl, 75 mM sodium citrate) at 42°C. Another example of high stringency conditions is 50% formamide, 5 x SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

The term "moderate stringency conditions" refers to those conditions which include the use of a

washing solution and hybridization conditions (e.g., temperature and ionic strength) less stringent than described above. An example of moderately stringent conditions are conditions such as overnight incubation  
5 at 37°C in a solution comprising: 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 µl/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled  
10 artisan will recognize how to adjust the temperature, ionic strength and other parameters as necessary to accommodate factors such as probe length and the like.

Recombinant DNA technology methods are set forth in Sambrook *et al.* (*Molecular Cloning: A  
15 Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)) and/or Ausubel *et al.*, eds., (*Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, NY (1994)) which are hereby incorporated by reference in their entirety.

20 The invention provides for isolated nucleic acid molecules encoding CRP1 and B7RP1 polypeptides. Also provided for are nucleic acid molecules which are fragments, allelic variants, splice variants, or are  
25 complementary in sequence to molecules encoding CRP1 and B7RP1 polypeptides. Nucleic acid molecules which are at least about 70% identical to molecules encoding CRP1 or B7RP1 or which hybridize to molecules encoding CRP1 or B7RP1 under moderate or high stringency  
30 conditions are also encompassed. The nucleic acid molecules may be cDNA, genomic DNA, RNA or a partially or totally synthetic nucleic acid molecule. In preferred embodiments, nucleic acid molecules of the invention are at least about 75%, 80%, 85%, 90% or 95%



identical to nucleic acid molecules encoding CRP1 or B7RP1.

A gene or cDNA encoding a CRP1 or B7RP1 polypeptide or fragment thereof may be obtained, for example, by hybridization screening or PCR amplification of a genomic or cDNA library. Probes or primers useful for screening the library can be generated based on sequence information for other known genes or gene fragments from the same or a related family of genes, for example, conserved motifs found in CRP1 or B7RP1 related polypeptides such as a conserved array of cysteine residues. In addition, where a gene encoding a CRP1 or B7RP1 polypeptide has been identified from one species, all or a portion of that gene may be used as a probe to identify homologous genes from other species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the CRP1 or B7RP1 gene.

Where oligonucleotide probes are used to screen cDNA or genomic libraries, one of the following two high stringency solutions may be used. The first of these is 6 X SSC with 0.05% sodium pyrophosphate at 35°C-62°C, with the temperature depending on the length of the oligonucleotide probe. For example, 14 base pair probes are washed at 35-40°C, 17 base pair probes are washed at 45-50°C, 20 base pair probes are washed at 52-57°C, and 23 base pair probes are washed at 57-63°C. The temperature can be increased 2-3°C where the background non-specific binding appears high. A second high stringency solution utilizes tetramethylammonium chloride (TMAC) for washing oligonucleotide probes. One stringent washing solution is 3 M TMAC, 50 mM Tris-HCl, pH 8.0, and 0.2% SDS. The washing temperature using this solution is a function of the length of the probe. For example, a 17 base pair probe is washed at about 45-50°C.

Another means to prepare a gene encoding a CRP1 or B7RP1 polypeptide or fragment thereof is to employ chemical synthesis using methods well known to the skilled artisan such as those described by Engels et al. (Agnew. Chem. Intl. Ed., 28:716-734 (1989)). These methods include, *inter alia*, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, DNA encoding a CRP1 or B7RP1 polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form a full length CRP1 or B7RP1 polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of a CRP1 or B7RP1 polypeptide, depending on whether the polypeptide produced in the host cell is designed to be secreted from that cell.

CRP1 or B7RP1 nucleic acid molecules, fragments, and/or derivatives that do not themselves encode polypeptides that are biologically active may nonetheless be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of CRP1 or B7RP1 DNA or corresponding RNA in mammalian tissue or bodily fluid samples.

In some cases, it may be desirable to prepare nucleic acid and/or amino acid variants of naturally occurring CRP1 or B7RP1 polypeptides. Nucleic acid variants may be produced using site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired point

5

10

15

35

| Parameter                  | Value            | Unit               |
|----------------------------|------------------|--------------------|
| Initial concentration      | 1.0              | g/L                |
| Initial pH                 | 7.0              |                    |
| Temperature                | 25               | °C                 |
| Time                       | 0-120            | min                |
| Agitation speed            | 150              | rpm                |
| Batch size                 | 100              | mL                 |
| Adsorbent dose             | 0.1-1.0          | g/L                |
| Adsorbent type             | Activated carbon |                    |
| Adsorbent surface area     | 1000             | m <sup>2</sup> /g  |
| Adsorbent pore volume      | 0.5              | cm <sup>3</sup> /g |
| Adsorbent density          | 1.5              | g/cm <sup>3</sup>  |
| Adsorbent particle size    | 0.25-0.5         | mm                 |
| Adsorbent batch            | 1                |                    |
| Adsorbent storage          | Room temperature |                    |
| Adsorbent treatment        | None             |                    |
| Adsorbent regeneration     | None             |                    |
| Adsorbent disposal         | Landfill         |                    |
| Adsorbent reuse            | None             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |
| Adsorbent biodegradability | Low              |                    |
| Adsorbent flammability     | Low              |                    |
| Adsorbent corrosivity      | Low              |                    |
| Adsorbent reactivity       | Low              |                    |
| Adsorbent compatibility    | High             |                    |
| Adsorbent safety           | High             |                    |
| Adsorbent handling         | Easy             |                    |
| Adsorbent storage          | Easy             |                    |
| Adsorbent disposal         | Easy             |                    |
| Adsorbent reuse            | Easy             |                    |
| Adsorbent cost             | 1.0              | \$/kg              |
| Adsorbent availability     | High             |                    |
| Adsorbent stability        | High             |                    |
| Adsorbent toxicity         | Low              |                    |

selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). The gene, cDNA or fragment thereof encoding the CRP1 or B7RP1 polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether the CRP1 or B7RP1 polypeptide or fragment thereof is to be glycosylated and/or phosphorylated. If so, yeast, insect, or mammalian host cells are preferable.

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and cloning and expression of inserted nucleotide sequences. Such sequences, referred to collectively as "flanking sequences", will include a promoter and other regulatory elements such as an enhancer(s), an origin of replication element, a transcriptional termination element, a complete intron sequence containing a donor and acceptor splice site, a signal peptide sequence, a ribosome binding site element, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these elements is discussed below. Optionally, the vector may contain a "tag" sequence, i.e., an oligonucleotide molecule located at the 5' or 3' end of a CRP1 or B7RP1 polypeptide coding sequence; the oligonucleotide molecule encodes polyHis (such as hexaHis), or other "tag" such as FLAG, HA (hemagglutinin Influenza virus) or myc for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as means for affinity purification of a CRP1 or B7RP1 polypeptide from the

host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from a purified CRP1 or B7RP1 polypeptide by various means such as using certain peptidases.

The human immunoglobulin hinge and Fc region may be fused at either the N-terminus or C-terminus of a CRP1 or B7RP1 polypeptide by one skilled in the art. The subsequent Fc-fusion protein can be purified by use of a Protein A affinity column. An immunoglobulin Fc region is known to exhibit a long pharmacokinetic half-life *in vivo* and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* compared to the unfused counterpart. Also, fusion to the Fc region allows for dimerization and/or multimerization of the molecule that may be useful for the bioactivity of some molecules.

The flanking sequence may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source), synthetic, or it may be the native CRP1 or B7RP1 nucleic acid flanking sequences. As such, the source of the flanking sequence may be any unicellular prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

The flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein other than CRP1 or B7RP1 nucleic acid flanking sequence will have been previously identified by mapping and/or by restriction

endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of the flanking sequence may be  
5 known. Here, the flanking sequence may be synthesized using the methods described above for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or  
10 by screening a genomic library with suitable oligonucleotide and/or flanking sequence fragments from the same or another species.

Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be  
15 isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion using one or more carefully selected enzymes to isolate the proper DNA fragment.  
20 After digestion, the desired fragment may be isolated by agarose gel purification, Qiagen® column or other methods known to the skilled artisan. Selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

25 The origin of replication element is typically a part of prokaryotic expression vectors purchased commercially, and aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be  
30 important for optimal expression of the CRP1 or B7RP1 polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector.

35 The transcription termination element is typically located 3' of the end of a CRP1 or B7RP1

5

10

25

35

|  | 1980 | 1981 | 1982 | 1983 | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | 2029 | 2030 | 2031 | 2032 | 2033 | 2034 | 2035 | 2036 | 2037 | 2038 | 2039 | 2040 | 2041 | 2042 | 2043 | 2044 | 2045 | 2046 | 2047 | 2048 | 2049 | 2050 | 2051 | 2052 | 2053 | 2054 | 2055 | 2056 | 2057 | 2058 | 2059 | 2060 | 2061 | 2062 | 2063 | 2064 | 2065 | 2066 | 2067 | 2068 | 2069 | 2070 | 2071 | 2072 | 2073 | 2074 | 2075 | 2076 | 2077 | 2078 | 2079 | 2080 | 2081 | 2082 | 2083 | 2084 | 2085 | 2086 | 2087 | 2088 | 2089 | 2090 | 2091 | 2092 | 2093 | 2094 | 2095 | 2096 | 2097 | 2098 | 2099 | 2100 | 2101 | 2102 | 2103 | 2104 | 2105 | 2106 | 2107 | 2108 | 2109 | 2110 | 2111 | 2112 | 2113 | 2114 | 2115 | 2116 | 2117 | 2118 | 2119 | 2120 | 2121 | 2122 | 2123 | 2124 | 2125 | 2126 | 2127 | 2128 | 2129 | 2130 | 2131 | 2132 | 2133 | 2134 | 2135 | 2136 | 2137 | 2138 | 2139 | 2140 | 2141 | 2142 | 2143 | 2144 | 2145 | 2146 | 2147 | 2148 | 2149 | 2150 | 2151 | 2152 | 2153 | 2154 | 2155 | 2156 | 2157 | 2158 | 2159 | 2160 | 2161 | 2162 | 2163 | 2164 | 2165 | 2166 | 2167 | 2168 | 2169 | 2170 | 2171 | 2172 | 2173 | 2174 | 2175 | 2176 | 2177 | 2178 | 2179 | 2180 | 2181 | 2182 | 2183 | 2184 | 2185 | 2186 | 2187 | 2188 | 2189 | 2190 | 2191 | 2192 | 2193 | 2194 | 2195 | 2196 | 2197 | 2198 | 2199 | 2200 | 2201 | 2202 | 2203 | 2204 | 2205 | 2206 | 2207 | 2208 | 2209 | 2210 | 2211 | 2212 | 2213 | 2214 | 2215 | 2216 | 2217 | 2218 | 2219 | 2220 | 2221 | 2222 | 2223 | 2224 | 2225 | 2226 | 2227 | 2228 | 2229 | 2230 | 2231 | 2232 | 2233 | 2234 | 2235 | 2236 | 2237 | 2238 | 2239 | 2240 | 2241 | 2242 | 2243 | 2244 | 2245 | 2246 | 2247 | 2248 | 2249 | 2250 | 2251 | 2252 | 2253 | 2254 | 2255 | 2256 | 2257 | 2258 | 2259 | 2260 | 2261 | 2262 | 2263 | 2264 | 2265 | 2266 | 2267 | 2268 | 2269 | 2270 | 2271 | 2272 | 2273 | 2274 | 2275 | 2276 | 2277 | 2278 | 2279 | 2280 | 2281 | 2282 | 2283 | 2284 | 2285 | 2286 | 2287 | 2288 | 2289 | 2290 | 2291 | 2292 | 2293 | 2294 | 2295 | 2296 | 2297 | 2298 | 2299 | 2300 | 2301 | 2302 | 2303 | 2304 | 2305 | 2306 | 2307 | 2308 | 2309 | 2310 | 2311 | 2312 | 2313 | 2314 | 2315 | 2316 | 2317 | 2318 | 2319 | 2320 | 2321 | 2322 | 2323 | 2324 | 2325 | 2326 | 2327 | 2328 | 2329 | 2330 | 2331 | 2332 | 2333 | 2334 | 2335 | 2336 | 2337 | 2338 | 2339 | 2340 | 2341 | 2342 | 2343 | 2344 | 2345 | 2346 | 2347 | 2348 | 2349 | 2350 | 2351 | 2352 | 2353 | 2354 | 2355 | 2356 | 2357 | 2358 | 2359 | 2360 | 2361 | 2362 | 2363 | 2364 | 2365 | 2366 | 2367 | 2368 | 2369 | 2370 | 2371 | 2372 | 2373 | 2374 | 2375 | 2376 | 2377 | 2378 | 2379 | 2380 | 2381 | 2382 | 2383 | 2384 | 2385 | 2386 | 2387 | 2388 | 2389 | 2390 | 2391 | 2392 | 2393 | 2394 | 2395 | 2396 | 2397 | 2398 | 2399 | 2400 | 2401 | 2402 | 2403 | 2404 | 2405 | 2406 | 2407 | 2408 | 2409 | 2410 | 2411 | 2412 | 2413 | 2414 | 2415 | 2416 | 2417 | 2418 | 2419 | 2420 | 2421 | 2422 | 2423 | 2424 | 2425 | 2426 | 2427 | 2428 | 2429 | 2430 | 2431 | 2432 | 2 |
|--|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|
|--|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|

the polypeptide from the host cell. A CRP1 or B7RP1 transmembrane domain is also inactivated by mutation or deletion to prevent attachment to the host membrane. Typically, the signal sequence is positioned in the coding region of a CRP1 or B7RP1 gene or cDNA, or directly at the 5' end of a CRP1 or B7RP1 gene coding region. Many signal sequences have been identified, and any of them that are functional in the selected host cell may be used in conjunction with a CRP1 or B7RP1 gene or cDNA. Therefore, the signal sequence may be homologous or heterologous to a CRP1 or B7RP1 gene or cDNA, and may be homologous or heterologous to a CRP1 or B7RP1 polypeptides gene or cDNA. Additionally, the signal sequence may be chemically synthesized using methods set forth above.

In most cases, secretion of the polypeptide from the host cell via the presence of a signal peptide will result in the removal of the amino terminal methionine from the polypeptide.

In many cases, transcription of a CRP1 or B7RP1 gene or cDNA is increased by the presence of one or more introns in the vector; this is particularly true where a CRP1 or B7RP1 polypeptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within a CRP1 or B7RP1 gene, especially where the gene used is a full length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron(s) may be obtained from another source. The position of the intron with respect to the 5' flanking sequence and a CRP1 or B7RP1 gene is generally important, as the intron must be transcribed to be effective. As such, where a CRP1 or B7RP1 gene inserted into the expression vector is a cDNA molecule, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA



Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pCDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15b (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), and pFastBacDual (Gibco/BRL, Grand Island, NY).

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). The host cell, when cultured under appropriate conditions, can synthesize a CRP1 or B7RP1 polypeptide which can

5 polypeptide can be purified using methods such as  
molecular sieve chromatography, affinity  
chromatography, and the like.

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO), human embryonic kidney (HEK) 293 or 293T-cells, or 3T3 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 and COS-7 cell lines, and the CV-1 cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Similarly useful as host cells are bacterial  
35 cells. For example, the various strains of *E. coli*

| Cognitive Function   |       | Mood    |       | Social Function |       | Quality of Life |       |
|----------------------|-------|---------|-------|-----------------|-------|-----------------|-------|
| Measure              | Score | Measure | Score | Measure         | Score | Measure         | Score |
| MMSE                 | 24.0  | BDI     | 10.0  | SF-36           | 45.0  | QoL             | 75.0  |
| MoCA                 | 22.0  | STAI    | 40.0  | WHOQOL          | 55.0  |                 |       |
| Trail Making Test    | 15.0  |         |       |                 |       |                 |       |
| Digit Span           | 12.0  |         |       |                 |       |                 |       |
| Block Design         | 18.0  |         |       |                 |       |                 |       |
| Verbal Fluency       | 10.0  |         |       |                 |       |                 |       |
| Stroop Test          | 12.0  |         |       |                 |       |                 |       |
| Digit Symbol         | 10.0  |         |       |                 |       |                 |       |
| Trail Making Test B  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test A  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test C  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test D  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test E  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test F  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test G  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test H  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test I  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test J  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test K  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test L  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test M  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test N  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test O  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test P  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test Q  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test R  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test S  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test T  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test U  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test V  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test W  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test X  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test Y  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test Z  | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AA | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AB | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AC | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AD | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AE | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AF | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AG | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AH | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AI | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AJ | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AK | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AL | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AM | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AN | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AO | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AP | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AQ | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AR | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AS | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AT | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AU | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AV | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AW | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AX | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AY | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test AZ | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BA | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BB | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BC | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BD | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BE | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BF | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BG | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BH | 15.0  |         |       |                 |       |                 |       |
| Trail Making Test BI | 15.0  |         |       |                 |       |                 |       |

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention.

"Transformation" or "transfection" of an expression vector into the selected host cell may be accomplished using such methods as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

The host cells transformed or transfected with an expression vector may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells are, for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum

and/or growth factors as required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary.

Typically, an antibiotic or other compound useful for selective growth of the transformed cells only is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin.

The amount of CRP1 or B7RP1 polypeptide produced in the host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If a CRP1 or B7RP1 polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. Polypeptides prepared in this way will typically not possess an amino terminal methionine, as it is removed during secretion from the cell. If, however, a CRP1 or B7RP1 polypeptide is not secreted from the host cells, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for gram negative bacteria host cells) and may have an amino terminal methionine.

Purification of a CRP1 or B7RP1 polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (CRP1

or B7RP1/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or myc (Invitrogen, Carlsbad, CA) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by  
5 passing the tagged polypeptide through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (*i.e.*, a monoclonal antibody specifically recognizing a CRP1 or B7RP1 polypeptide). For example, polyhistidine binds  
10 with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of CRP1 or B7RP1/polyHis. (See for example, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Section  
15 10.11.8, John Wiley & Sons, New York (1993)).

Where a CRP1 or B7RP1 polypeptide is prepared without a tag attached, and no antibodies are available, other well known procedures for purification can be used. Such procedures include, without  
20 limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of  
25 these techniques may be combined to achieve increased purity.

If it is anticipated that a CRP1 or B7RP1 polypeptide will be found primarily intracellularly, the intracellular material (including inclusion  
30 bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization,  
35 and/or sonication followed by centrifugation.

If a CRP1 or B7RP1 polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate a CRP1 or B7RP1 polypeptide, isolation may be accomplished using standard methods such as those set forth below and in Marston et al. (*Meth. Enz.*, 182:264-275 (1990)). In some cases, a CRP1 or B7RP1 polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological activity. Such methods include contacting the solubilized polypeptide with a solution having a pH usually above 7 and in the presence of a particular concentration of an appropriate chaotrope. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent and the corresponding oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride,

dithiothreitol (DTT)/dithiane DTT, 2-mercaptoethanol (bME)/dithio-b(ME). In many instances a cosolvent is necessary to increase the efficiency of the refolding and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, and arginine.

CRP1 or B7RP1 polypeptides, fragments, and/or derivatives thereof may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield *et al.*, (*J. Am. Chem. Soc.*, 85:2149 (1963)), Houghten *et al.* (*Proc Natl Acad. Sci. USA*, 82:5132 (1985)), and Stewart and Young (*Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL (1984)). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized CRP1 or B7RP1 polypeptides or fragments may be oxidized using methods set forth in these references to form disulfide bridges. CRP1 or B7RP1 polypeptides or fragments are expected to have biological activity comparable to CRP1 or B7RP1 polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with recombinant or natural CRP1 or B7RP1 polypeptide.

### Polypeptides

The term "CRP1 or B7RP1 polypeptide" refers to a polypeptide having the amino acid sequence of Figure 1A (SEQ ID NO:2), Figure 2A (SEQ ID NO:7) or Figure 3A (SEQ ID NO:12) and all related polypeptides described herein. Related polypeptides includes allelic variants, splice variants, fragments, derivatives, substitution, deletion, and insertion

5

10

30

[illegible]



| Table 1. Demographic characteristics of the study population |             |
|--|-------------|
| Age (years)  | 50.0 ± 10.0 |
| Gender   |             |
| Male   | 50.0        |
| Female   | 50.0        |
| Marital status   |             |
| Married  | 50.0        |
| Single   | 50.0        |
| Divorced   | 50.0        |
| Widowed  | 50.0        |
| Education  |             |
| High school  | 50.0        |
| College  | 50.0        |
| Postgraduate   | 50.0        |
| Occupation   |             |
| Professional   | 50.0        |
| Managerial   | 50.0        |
| Technical  | 50.0        |
| Service  | 50.0        |
| Unemployed   | 50.0        |
| Retired  | 50.0        |
| Income (USD/month)   |             |
| < 1000   | 50.0        |
| 1000-2000  | 50.0        |
| 2000-3000  | 50.0        |
| > 3000   | 50.0        |
| Health status  |             |
| Good   | 50.0        |
| Fair   | 50.0        |
| Poor   | 50.0        |
| Smoking status   |             |
| Smoker   | 50.0        |
| Non-smoker   | 50.0        |
| Alcohol consumption  |             |
| Regular  | 50.0        |
| Occasional   | 50.0        |
| Never  | 50.0        |

The term "ortholog" refers to a polypeptide that corresponds to a polypeptide identified from a species. For example, mouse and human B7RP1 polypeptides are considered orthologs.

The term "isolated polypeptide" refers to a polypeptide that is free from at least one contaminating polypeptide that is found in its natural environment, and preferably substantially free from any other contaminating mammalian polypeptides.

The term "identity," as known in the art, is  
35 a relationship between the sequences of two or more  
nucleic acid molecules or two or more polypeptides, as

5

10

35

Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part 1*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., *Nucleic Acids Research* 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215:403-410 (1990). The BLAST X program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (*BLAST Manual*, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215:403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

By way of example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated

as 3 X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff et al., in: Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for the PAM250 comparison matrix; see Henikoff et al., *Proc. Natl. Acad. Sci USA*, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48:443-453 (1970)

Comparison matrix: BLOSUM 62 from Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

Threshold of Similarity: 0

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48:443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

5                   The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

10                   Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used by those of skill in the art, including those set forth in the Program Manual, Wisconsin Package, Version 9,  
15                   September, 1997. The particular choices to be made will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between pairs of sequences (in which case GAP or BestFit are  
20                   generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

                  Polypeptides that are at least about 70 percent identical will typically have one or more amino  
25                   acid substitutions, deletions, and/or additions as compared to a wild type CRP1 or B7RP1 polypeptide. In preferred embodiment, polypeptides will have about 75%, 80%, 85%, 90% or 95% identity to CRP1 or B7RP1 polypeptides. Usually, the substitutions of the native  
30                   residue will be either alanine, or a conservative amino acid so as to have little or no effect on the overall net charge, polarity, or hydrophobicity of the polypeptide. Conservative substitutions are set forth in Table I below.

35

Table I  
Conservative Amino Acid Substitutions

|                  |               |
|------------------|---------------|
| Basic:           | arginine      |
|                  | lysine        |
|                  | histidine     |
| Acidic:          | glutamic acid |
|                  | aspartic acid |
| Uncharged Polar: | glutamine     |
|                  | asparagine    |
|                  | serine        |
|                  | threonine     |
|                  | tyrosine      |
|                  | phenylalanine |
|                  | tryptophan    |
| Non-Polar:       | cysteine      |
|                  | glycine       |
|                  | alanine       |
|                  | valine        |
|                  | proline       |
|                  | methionine    |
|                  | leucine       |
|                  | norleucine    |
|                  | isoleucine    |

5

CRP1 or B7RP1 polypeptide derivatives are provided by the invention. In one embodiment, chemically modified CRP1 or B7RP1 polypeptide compositions in which CRP1 or B7RP1 polypeptides are  
10 linked to a polymer are included within the scope of the present invention. The polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment.  
15 The polymer selected is usually modified to have a

single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled as provided for in the present methods. The polymer  
5 may be of any molecular weight, and may be branched or unbranched. Included within the scope of the invention is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

10 The water soluble polymer or mixture thereof may be selected from the group consisting of, for example, polyethylene glycol (PEG), monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl  
15 pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol.

For the acylation reactions, the polymer(s)  
20 selected should have a single reactive ester group. For reductive alkylation, the polymer(s) selected should have a single reactive aldehyde group. A preferred reactive aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C1-C10  
25 alkoxy or aryloxy derivatives thereof (see U.S. Patent No. 5,252,714).

Pegylation of CRP1 or B7RP1 polypeptides may be carried out by any of the pegylation reactions known in the art, as described for example in the following  
30 references: *Focus on Growth Factors* 3: 4-10 (1992); EP 0 154 316; and EP 0 401 384. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble  
35 polymer) as described below.

| Table 1. Demographic characteristics of the study population |                |
|--|----------------|
| Age (years)  | Mean (SD)      |
| 18-24  | 20.5 (2.5)     |
| 25-34  | 29.5 (4.5)     |
| 35-44  | 39.5 (5.5)     |
| 45-54  | 49.5 (6.5)     |
| 55-64  | 59.5 (7.5)     |
| 65-74  | 69.5 (8.5)     |
| 75-84  | 79.5 (9.5)     |
| 85-94  | 89.5 (10.5)    |
| 95-104   | 99.5 (11.5)    |
| 105-114  | 109.5 (12.5)   |
| 115-124  | 119.5 (13.5)   |
| 125-134  | 129.5 (14.5)   |
| 135-144  | 139.5 (15.5)   |
| 145-154  | 149.5 (16.5)   |
| 155-164  | 159.5 (17.5)   |
| 165-174  | 169.5 (18.5)   |
| 175-184  | 179.5 (19.5)   |
| 185-194  | 189.5 (20.5)   |
| 195-204  | 199.5 (21.5)   |
| 205-214  | 209.5 (22.5)   |
| 215-224  | 219.5 (23.5)   |
| 225-234  | 229.5 (24.5)   |
| 235-244  | 239.5 (25.5)   |
| 245-254  | 249.5 (26.5)   |
| 255-264  | 259.5 (27.5)   |
| 265-274  | 269.5 (28.5)   |
| 275-284  | 279.5 (29.5)   |
| 285-294  | 289.5 (30.5)   |
| 295-304  | 299.5 (31.5)   |
| 305-314  | 309.5 (32.5)   |
| 315-324  | 319.5 (33.5)   |
| 325-334  | 329.5 (34.5)   |
| 335-344  | 339.5 (35.5)   |
| 345-354  | 349.5 (36.5)   |
| 355-364  | 359.5 (37.5)   |
| 365-374  | 369.5 (38.5)   |
| 375-384  | 379.5 (39.5)   |
| 385-394  | 389.5 (40.5)   |
| 395-404  | 399.5 (41.5)   |
| 405-414  | 409.5 (42.5)   |
| 415-424  | 419.5 (43.5)   |
| 425-434  | 429.5 (44.5)   |
| 435-444  | 439.5 (45.5)   |
| 445-454  | 449.5 (46.5)   |
| 455-464  | 459.5 (47.5)   |
| 465-474  | 469.5 (48.5)   |
| 475-484  | 479.5 (49.5)   |
| 485-494  | 489.5 (50.5)   |
| 495-504  | 499.5 (51.5)   |
| 505-514  | 509.5 (52.5)   |
| 515-524  | 519.5 (53.5)   |
| 525-534  | 529.5 (54.5)   |
| 535-544  | 539.5 (55.5)   |
| 545-554  | 549.5 (56.5)   |
| 555-564  | 559.5 (57.5)   |
| 565-574  | 569.5 (58.5)   |
| 575-584  | 579.5 (59.5)   |
| 585-594  | 589.5 (60.5)   |
| 595-604  | 599.5 (61.5)   |
| 605-614  | 609.5 (62.5)   |
| 615-624  | 619.5 (63.5)   |
| 625-634  | 629.5 (64.5)   |
| 635-644  | 639.5 (65.5)   |
| 645-654  | 649.5 (66.5)   |
| 655-664  | 659.5 (67.5)   |
| 665-674  | 669.5 (68.5)   |
| 675-684  | 679.5 (69.5)   |
| 685-694  | 689.5 (70.5)   |
| 695-704  | 699.5 (71.5)   |
| 705-714  | 709.5 (72.5)   |
| 715-724  | 719.5 (73.5)   |
| 725-734  | 729.5 (74.5)   |
| 735-744  | 739.5 (75.5)   |
| 745-754  | 749.5 (76.5)   |
| 755-764  | 759.5 (77.5)   |
| 765-774  | 769.5 (78.5)   |
| 775-784  | 779.5 (79.5)   |
| 785-794  | 789.5 (80.5)   |
| 795-804  | 799.5 (81.5)   |
| 805-814  | 809.5 (82.5)   |
| 815-824  | 819.5 (83.5)   |
| 825-834  | 829.5 (84.5)   |
| 835-844  | 839.5 (85.5)   |
| 845-854  | 849.5 (86.5)   |
| 855-864  | 859.5 (87.5)   |
| 865-874  | 869.5 (88.5)   |
| 875-884  | 879.5 (89.5)   |
| 885-894  | 889.5 (90.5)   |
| 895-904  | 899.5 (91.5)   |
| 905-914  | 909.5 (92.5)   |
| 915-924  | 919.5 (93.5)   |
| 925-934  | 929.5 (94.5)   |
| 935-944  | 939.5 (95.5)   |
| 945-954  | 949.5 (96.5)   |
| 955-964  | 959.5 (97.5)   |
| 965-974  | 969.5 (98.5)   |
| 975-984  | 979.5 (99.5)   |
| 985-994  | 989.5 (100.5)  |
| 995-1004   | 999.5 (101.5)  |
| 1005-1014  | 1009.5 (102.5) |
| 1015-1024  | 1019.5 (103.5) |
| 1025-1034  | 1029.5 (104.5) |
| 1035-1044  | 1039.5 (105.5) |
| 1045-1054  | 1049.5 (106.5) |
| 1055-1064  | 1059.5 (107.5) |
| 1065-1074  | 1069.5 (108.5) |
| 1075-1084  | 1079.5 (109.5) |
| 1085-1094  | 1089.5 (110.5) |
| 1095-1104  | 1099.5 (111.5) |
| 1105-1114  | 1109.5 (112.5) |
| 1115-1124  | 1119.5 (113.5) |
| 1125-1134  |                |

Generally, conditions which may be alleviated or modulated by administration of CRP1 or B7RP1 polymer conjugates include those described herein for non-  
25 conjugated CRP1 or B7RP1 polypeptides. However, the conjugated disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased  
30 half-life, as compared to the non-derivatized molecules.

CRP1 or B7RP1 polypeptides, fragments  
variants, and derivatives, may be employed alone,  
together, or in combination with other pharmaceutical  
compositions. CRP1 or B7RP1 polypeptides, fragments,  
35 variants, and derivatives may be used in combination  
with cytokines, growth factors, antibiotics, anti-



inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

5 The invention provides for selective binding agents of CRP1 or B7RP1. A selective binding agent refers to a molecule having specificity for CRP1 or B7RP1 and may include a protein, peptide, nucleic acid, carbohydrate, lipid or small molecular weight compound. A selective binding agent interacts either with CRP1 or  
10 B7RP1 and in turn regulates the binding of CRP1 to B7RP1. In one embodiment, a selective binding agent partially or completely blocks the binding of CRP1 to B7RP1 and partially or completely inhibits at least one biological activity of CRP1 or B7RP1, such as immune  
15 costimulatory activity. In another embodiment, the selective binding agent is an antibody. The antibody may be immunoreactive with either CRP1 or B7RP1 and is preferably immunoreactive with B7RP1. In yet another embodiment of the invention, an antibody reactive with  
20 B7RP1 binds to an epitope on B7RP1 such that binding to CRP1 is partially or completely blocked and at least one biological activity of B7RP1, such as immune costimulatory activity, is partially or completely inhibited. The term partially inhibited means that at  
25 least a detectable level of inhibition has occurred. The term completely inhibited means that no further increase in inhibition has occurred.

CRP1 or B7RP1 polypeptides, fragments, variants, and/or derivatives may be used to prepare  
30 antibodies using methods known in the art. Thus, antibodies that react with the CRP1 or B7RP1 polypeptides, as well as reactive fragments of such antibodies, are also contemplated as within the scope of the present invention. The antibodies may be  
35 polyclonal, monoclonal, recombinant, chimeric, single-chain and/or bispecific. Typically, the antibody or

fragment thereof will either be of human origin, or will be "humanized", i.e., prepared so as to prevent or minimize an immune reaction to the antibody when administered to a patient. The antibody fragment may  
5 be any fragment that is reactive with CRP1 and B7RP1 polypeptides of the present invention, such as, Fab, Fab', etc. Also provided by this invention are the hybridomas generated by presenting any CRP1 or B7RP1 polypeptide or fragments thereof as an antigen to a  
10 selected mammal, followed by fusing cells (e.g., spleen cells) of the mammal with certain cancer cells to create immortalized cell lines by known techniques. The methods employed to generate such cell lines and antibodies directed against all or portions of a human  
15 CRP1 or B7RP1 polypeptide of the present invention are also encompassed by this invention.

Monoclonal antibodies of the invention include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or  
20 homologous to corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chains(s) is identical with or homologous to corresponding sequence in antibodies  
25 derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison, et al., Proc. Natl. Acad. Sci. 81, 6851-6855  
30 (1985)).

In a preferred embodiment, the chimeric anti-CRP1 or B7RP1 antibody is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has  
35 one or more amino acid residues introduced into it from

1 a source which is non-human. Humanization can be  
performed following methods known in the art (Jones, et  
al., Nature 321, 522-525 (1986); Riechmann, et al.,  
Nature, 332, 323-327 (1988); Verhoeyen, et al., Science  
5 239, 1534-1536 (1988)), by substituting rodent  
complementarily-determining regions (CDRs) for the  
corresponding regions of a human antibody.

Also encompassed by the invention are fully  
human anti-CRP1 or anti-B7RP1 antibodies. Such  
10 antibodies may be produced by immunization with a CRP1  
or B7RP1 antigen of transgenic animals (e.g., mice)  
that are capable of producing a repertoire of human  
antibodies in the absence of endogenous immunoglobulin  
production. See, for example, Jakobovits, et al.,  
15 Proc. Natl. Acad. Sci. 90, 2551-2555 (1993);  
Jakobovits, et al., Nature 362, 255-258 (1993). Human  
antibodies can also be produced in phage-display  
libraries (Hoogenboom, et al., J. Mol. Biol. 227, 381  
(1991); Marks, et al., J. Mol. Biol. 222, 581 (1991).

20 Selective binding agents of the invention may  
be used to regulate the binding of CRP1 to B7RP1 and  
regulate at least one biological activity mediated by  
CRP1 and B7RP1 such as immune co-stimulation. An  
example of such selective binding agents are antibodies  
25 immunoreactive with either CRP1 or B7RP1. The  
antibodies may be used therapeutically, such as to  
inhibit binding of the CRP1 and B7RP1 polypeptide to  
its binding partner. The antibodies may further be  
used for *in vivo* and *in vitro* diagnostic purposes, such  
30 as in labeled form to detect the presence of CRP1 and  
B7RP1 polypeptide in a body fluid or cell sample.

#### Pharmaceutical Compositions and Administration

35 Pharmaceutical compositions of CRP1 or B7RP1  
polypeptides are within the scope of the present  
invention. Such compositions may comprise a

therapeutically effective amount of the polypeptide or fragments, variants, or derivatives in admixture with a pharmaceutically acceptable carrier. In preferred embodiments, pharmaceutical compositions comprise CRP1 or B7RP1 polypeptides as soluble forms which comprise part or all of a CRP1 or B7RP1 extracellular domain. Typically, a CRP1 and B7RP1 polypeptide therapeutic compound will be administered in the form of a composition comprising purified polypeptide, fragment, variant, or derivative in conjunction with one or more physiologically acceptable carriers, excipients, or diluents. The carrier material may be water for injection, preferably supplemented with other materials common in solutions for administration to mammals. Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. Preferably, the product is formulated as a lyophilizate using appropriate excipients (e.g., sucrose). Other standard carriers, diluents, and excipients may be included as desired. Other exemplary compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor.

CRP1 or B7RP1 pharmaceutical compositions can be administered parenterally. Alternatively, the compositions may be administered intravenously or subcutaneously. When systemically administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such pharmaceutically acceptable protein solutions, with due regard to pH, isotonicity, stability and the like, is within the skill of the art.

Therapeutic formulations of CRP1 and B7RP1 polypeptide compositions useful for practicing the present invention may be prepared for storage by mixing

the selected composition having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company (1990)) in the form of a lyophilized cake or an aqueous solution. Acceptable carriers, excipients or stabilizers are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

An effective amount of a CRP1 or B7RP1 polypeptide composition(s) to be employed therapeutically will depend, for example, upon the therapeutic objectives such as the indication for which the CRP1 and B7RP1 polypeptide is being used, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage may range from about 0.1  $\mu\text{g/kg}$  to up to 100 mg/kg or more, depending on the factors mentioned above. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two

or more doses (which may or may not contain the same amount of a CRP1 or B7RP1 polypeptide) over time, or as a continuous infusion via implantation device or catheter.

5           As further studies are conducted, information  
will emerge regarding appropriate dosage levels for  
treatment of various conditions in various patients,  
and the ordinary skilled worker, considering the  
therapeutic context, the type of disorder under  
10 treatment, the age and general health of the recipient,  
will be able to ascertain proper dosing.

The CRP1 or B7RP1 polypeptide composition to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration ordinarily will be stored in lyophilized form or in solution.

Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

25           The route of administration of the  
composition is in accord with known methods, e.g. oral,  
injection or infusion by intravenous, intraperitoneal,  
intracerebral (intraparenchymal),  
intracerebroventricular, intramuscular, intraocular,  
30 intraarterial, or intralesional routes, or by sustained  
release systems or implantation device which may  
optionally involve the use of a catheter. Where  
desired, the compositions may be administered  
continuously by infusion, bolus injection or by  
35 implantation device.

Alternatively or additionally, the composition may be administered locally via implantation into the affected area of a membrane, sponge, or other appropriate material on to which CRP1  
5 and B7RP1 polypeptide has been absorbed.

Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of a CRP1 or B7RP1 polypeptide may be directly through the device via bolus, or via  
10 continuous administration, or via catheter using continuous infusion. A CRP1 or B7RP1 polypeptide may be administered in a sustained release formulation or preparation. Suitable examples of sustained-release preparations include semipermeable polymer matrices in  
15 the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al*, *Biopolymers*, 22: 547-556  
20 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15: 167-277 (1981)) and Langer, *Chem. Tech.*, 12: 98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release  
25 compositions also may include liposomes, which can be prepared by any of several methods known in the art (e.g., Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949).

30 In some cases, it may be desirable to use CRP1 or B7RP1 polypeptide compositions in an *ex vivo* manner. Here, cells, tissues, or organs that have been removed from the patient are exposed to a CRP1 or B7RP1 polypeptide compositions after which the cells, tissues  
35 and/or organs are subsequently implanted back into the patient.

00321 024260

In other cases, a CRP1 or B7RP1 polypeptide may be delivered through implanting into patients certain cells that have been genetically engineered, using methods such as those described herein, to

5 express and secrete the polypeptides, fragments, variants, or derivatives. Such cells may be animal or human cells, and may be derived from the patient's own tissue or from another source, either human or non-human. Optionally, the cells may be immortalized.

10 However, in order to decrease the chance of an immunological response, it is preferred that the cells be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or

15 membranes that allow release of the protein product(s) but prevent destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Methods used for membrane encapsulation of

20 cells are familiar to the skilled artisan, and preparation of encapsulated cells and their implantation in patients may be accomplished without undue experimentation. See, e.g., U.S. Patent Nos. 4,892,538; 5,011,472; and 5,106,627. A system for

25 encapsulating living cells is described in PCT WO 91/10425 (Aebischer et al.). Techniques for formulating a variety of other sustained or controlled delivery means, such as liposome carriers, bio-erodible particles or beads, are also known to those in the art,

30 and are described, for example, in U.S. Patent No. 5,653,975. The cells, with or without encapsulation, may be implanted into suitable body tissues or organs of the patient.

As discussed above, it may be desirable to

35 treat cell preparations with one or more CRP1 or B7RP1 polypeptides, variants, derivatives and/or fragments.



This can be accomplished by exposing, for example, cells comprising T-cells, such as bone marrow cells, to the polypeptide, variant, derivative, or fragment directly, where it is in a form that is permeable to the cell membrane. For example, cells comprising T-cells may be exposed to a B7RP1 polypeptide in order to activate T-cell function and the cells so treated are implanted in the patient.

Alternatively, gene therapy can be employed. One manner in which gene therapy can be applied is to use a CRP1 or B7RP1 gene (either genomic DNA, cDNA, and/or synthetic DNA encoding a CRP1 or B7RP1 polypeptide, or a fragment, variant, or derivative thereof) which may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct". The promoter may be homologous or heterologous to the endogenous CRP1 or B7RP1 gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include, as required, DNA molecules designed for site-specific integration (e.g., endogenous flanking sequences useful for homologous recombination), tissue-specific promoter, enhancer(s) or silencer(s), DNA molecules capable of providing a selective advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (as, for example, for cell targeting) cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as factors to enable vector manufacture.

This gene therapy DNA construct can then be introduced into the patient's cells (either *ex vivo* or *in vivo*). One means for introducing the gene therapy DNA construct is via viral vectors. Suitable viral

[illegible]

Alternative means to deliver gene therapy DNA constructs to a patient's cells without the use of viral vectors include, without limitation, liposome-mediated transfer, direct injection of naked DNA, receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (e.g., "gene gun"). See U.S. Patent Nos. 4,970,154, WO 96/40958, 5,679,559, 5,676,954, and 5,593,875.

Another means to increase endogenous CRP1 or B7RP1 polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the CRP1 or B7RP1 polypeptide promoter, where the enhancer element(s) can serve to increase transcriptional activity of a CRP1 or B7RP1 polypeptide gene. The enhancer element(s) used will be selected based on the tissue in which one desires to activate the gene(s); enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a CRP1 or B7RP1 polypeptide is to be "turned on" in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of

5

10

15

20

25

30

35

via a viral vector as described above. Typically, integration of the construct into the genomic DNA of the cells will be via homologous recombination, where the 5' and 3' flanking DNA sequences in the promoter  
5 construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

Other gene therapy methods may also be employed where it is desirable to inhibit one or more  
10 CRP1 or B7RP1 polypeptides. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of a selected CRP1 or B7RP1 polypeptide gene(s) can be introduced into the cell. Typically, each such antisense molecule will be  
15 complementary to the start site (5' end) of each selected CRP1 or B7RP1 gene. When the antisense molecule then hybridizes to the corresponding CRP1 or B7RP1 polypeptide mRNA, translation of this mRNA is prevented.

20 Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more CRP1 or B7RP1 polypeptides. In this situation, the DNA encoding a mutant full length or truncated polypeptide of each selected CRP1 or B7RP1 polypeptide can be  
25 prepared and introduced into the cells of a patient using either viral or non-viral methods as described above. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

30

#### Agonists and Antagonists

The invention also provides for agonists and antagonists of CRP1 or B7RP1 which regulate the activity of either or both molecules. A CRP1 or B7RP1  
35 agonist will stimulate or enhance at least one activity of CRP1 or B7RP1. A CRP1 or B7RP1 antagonist will

| Variable             | Mean | Standard Deviation | Minimum | Maximum |
|----------------------|------|--------------------|---------|---------|
| Age                  | 35.2 | 12.5               | 20      | 65      |
| Gender               | 0.45 | 0.50               | 0       | 1       |
| Marital Status       | 0.60 | 0.49               | 0       | 1       |
| Education            | 12.5 | 2.5                | 9       | 16      |
| Income               | 3500 | 1500               | 1000    | 8000    |
| Health               | 0.75 | 0.43               | 0       | 1       |
| Smoking              | 0.20 | 0.40               | 0       | 1       |
| Alcohol              | 0.10 | 0.30               | 0       | 1       |
| Exercise             | 0.30 | 0.46               | 0       | 1       |
| Stress               | 0.50 | 0.50               | 0       | 1       |
| Sleep                | 0.60 | 0.49               | 0       | 1       |
| Appetite             | 0.70 | 0.46               | 0       | 1       |
| Mood                 | 0.65 | 0.48               | 0       | 1       |
| Energy               | 0.70 | 0.46               | 0       | 1       |
| Concentration        | 0.60 | 0.49               | 0       | 1       |
| Memory               | 0.65 | 0.48               | 0       | 1       |
| Emotion              | 0.60 | 0.49               | 0       | 1       |
| Behavior             | 0.60 | 0.49               | 0       | 1       |
| Thought              | 0.60 | 0.49               | 0       | 1       |
| Feeling              | 0.60 | 0.49               | 0       | 1       |
| Perception           | 0.60 | 0.49               | 0       | 1       |
| Attention            | 0.60 | 0.49               | 0       | 1       |
| Intuition            | 0.60 | 0.49               | 0       | 1       |
| Imagination          | 0.60 | 0.49               | 0       | 1       |
| Reasoning            | 0.60 | 0.49               | 0       | 1       |
| Logic                | 0.60 | 0.49               | 0       | 1       |
| Analysis             | 0.60 | 0.49               | 0       | 1       |
| Synthesis            | 0.60 | 0.49               | 0       | 1       |
| Evaluation           | 0.60 | 0.49               | 0       | 1       |
| Comparison           | 0.60 | 0.49               | 0       | 1       |
| Classification       | 0.60 | 0.49               | 0       | 1       |
| Organization         | 0.60 | 0.49               | 0       | 1       |
| Planning             | 0.60 | 0.49               | 0       | 1       |
| Problem Solving      | 0.60 | 0.49               | 0       | 1       |
| Decision Making      | 0.60 | 0.49               | 0       | 1       |
| Communication        | 0.60 | 0.49               | 0       | 1       |
| Interpersonal Skills | 0.60 | 0.49               | 0       | 1       |
| Teamwork             | 0.60 | 0.49               | 0       | 1       |
| Leadership           | 0.60 | 0.49               | 0       | 1       |
| Management           | 0.60 | 0.49               | 0       | 1       |
| Organization Skills  | 0.60 | 0.49               | 0       | 1       |
| Time Management      | 0.60 | 0.49               | 0       | 1       |
| Resource Management  | 0.60 | 0.49               | 0       | 1       |
| Conflict Resolution  | 0.60 | 0.49               | 0       | 1       |
| Stress Management    | 0.60 | 0.49               | 0       | 1       |
| Emotional Regulation | 0.60 | 0.49               | 0       | 1       |
| Self-Motivation      | 0.60 | 0.49               | 0       | 1       |
| Goal Setting         | 0.60 | 0.49               | 0       | 1       |
| Self-Reflection      | 0.60 | 0.49               | 0       | 1       |
| Personal Growth      | 0.60 | 0.49               | 0       | 1       |
| Life Satisfaction    | 0.60 | 0.49               | 0       | 1       |
| Overall Well-Being   | 0.60 | 0.49               | 0       | 1       |

10

15

20

25

30

35

As an alternative to radiolabelling, CRP1 or B7RP1 may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horse radish peroxidase [HRP] or alkaline phosphatase [AP], that can be detected colorometrically, or by fluorescent tagging of streptavidin. An antibody directed to CRP1 or B7RP1 that is conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP

CRP1 and B7RP1 may also be immobilized by attachment to agarose beads, acrylic beads or other types of such inert substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound; after incubation, the beads can be precipitated by centrifugation, and the amount of binding between CRP1 and B7RP1 can be assessed using the methods described above. Alternatively, the substrate-protein complex can be immobilized in a column and the test molecule and complementary protein passed over the column. Formation of a complex between CRP1 and B7RP1 can then be assessed using any of the techniques set forth above, i.e., radiolabelling, antibody binding, or the like.

Another type of in vitro assay that is useful for identifying a test molecule which increases or decreases formation of an CRP1/B7RP1 complex is a surface plasmon resonance detector system such as the Biacore assay system (Pharmacia, Piscataway, NJ). The Biacore system may be carried out using the manufacturer's protocol. This assay essentially involves covalent binding of either CRP1 or B7RP1 to a dextran-coated sensor chip which is located in a detector. The test compound and the other complementary protein can then be injected into the

chamber containing the sensor chip either simultaneously or sequentially and the amount of complementary protein that binds can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test compounds together for use in increasing or decreasing formation of a CRP1/B7RP1 complex. In these cases, the assays set forth above can be readily modified by adding such additional test compound(s) either simultaneously with, or subsequently to, the first test compound. The remainder of steps in the assay are as set forth above.

In vitro assays such as those described above may be used advantageously to screen rapidly large numbers of compounds for effects on complex formation by CRP1 and B7RP1. The assays may be automated to screen compounds generated in phage display, synthetic peptide and chemical synthesis libraries.

Compounds which increase or decrease complex formation of CRP1 and B7RP1 may also be screened in cell culture using cells and cell lines expressing either polypeptide. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of B7RP1 to cells expressing CRP1 on the surface is evaluated in the presence or absence of test molecules and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to B7RP1. Cell culture assays may be used advantageously to further evaluate compounds that score positive in protein binding assays described above.

#### Therapeutic Uses

Polypeptides of the invention, and agonists and antagonists thereof, may be used to regulate T-cell function. Agonists and antagonists include those molecules which regulate CRP1 and/or B7RP1 activity and either increase or decrease at least one activity of a CRP1 or B7RP1 protein such as one activity associated with T-cell functions, for example, T-cell activation. Agonists or antagonists may be co-factors, such as a protein, peptide, carbohydrate, lipid, or small molecular weight molecule, which interact with either CRP1 or B7RP1 and thereby regulate their activity. Potential polypeptide agonists or antagonists include antibodies that react with either soluble or membrane-bound forms of CRP1 or B7RP1 which comprise part or all of the extracellular domains of the said proteins. Molecules that regulate CRP1 or B7RP1 expression typically include nucleic acids encoding CRP1 or B7RP1 protein that can act as anti-sense regulators of expression.

CRP1 or B7RP1 polypeptides, and agonists and antagonists thereof, may be used in the treatment of autoimmune disease, graft survival, immune cell activation for inhibiting tumor cell growth, T-cell dependent B-cell mediated diseases, and cancer gene immunotherapy. In one embodiment, antagonists or inhibitors of CRP1 and/or B7RP1 function may be beneficial to alleviate symptoms in diseases with chronic immune cell dysfunction. Autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, immune thrombocytopenic purpura (ITP), and psoriasis, may be treated with antagonists or inhibitors of CRP1/B7RP1. In addition, chronic inflammatory diseases, such as inflammatory bowel disease (Crohn's disease and ulcerative colitis), Grave's disease, Hashimoto's thyroiditis, and diabetes mellitus, may also be treated with inhibitors to



CRP1/B7RP1. As described in Example 18, CRP1-Fc inhibits and B7RP1-Fc enhances, the onset of disease in a rodent rheumatoid arthritis disease model. These opposite effects in this model support an agonistic role for the B7RP1-Fc protein and an antagonistic role for the CRP1-Fc protein. The results also illustrate how T-cell responses can be regulated by manipulation of this pathway and the significance of this pathway in the progression of rheumatoid arthritis. In addition, as described in Example 19, expression of B7RP1-Fc *in vivo* stimulates an inflammatory bowel disease (IBD) phenotype in transgenic mice. This example supports the role for B7RP1/CRP1 in the development of inflammation in the intestine. Therefore, antagonists of the B7RP1/CRP1 pathway may be used to treat human IBD.

Antagonists of CRP1 or B7RP1 may be used as immunosuppressive agents for bone marrow and organ transplantation and may be used to prolong graft survival. Such antagonists may provide significant advantages over existing treatment. Bone marrow and organ transplantation therapy must contend with T-cell mediated rejection of the foreign cells or tissue by the host. Present therapeutic regimens for inhibiting T-cell mediated rejection involve treatment with the drugs cyclosporine or FK506. While drugs are effective, patients suffer from serious side effects, including hepatotoxicity, nephrotoxicity, and neurotoxicity. The target for the cyclosporin/FK506 class of therapeutics is calcineurin, a phosphatase with ubiquitous expression. Since CRP1 expression is restricted to T-cells, inhibitors of CRP1 or B7RP1 may lack the severe side effects observed with the use of the present immunotherapeutic agents.

Antagonists of CRP1 or B7RP1 may be used as immunosuppressive agents for autoimmune disorders, such

| Variable  | Mean | SD   | Min | Max |
|---|------|------|-----|-----|
| Age   | 34.5 | 10.2 | 18  | 65  |
| Gender  | 0.45 | 0.50 | 0   | 1   |
| Marital status  | 0.60 | 0.49 | 0   | 1   |
| Education   | 12.5 | 1.5  | 9   | 16  |
| Income  | 15.2 | 8.5  | 5   | 35  |
| Health status   | 0.75 | 0.43 | 0   | 1   |
| Stress level  | 2.8  | 1.2  | 1   | 5   |
| Life satisfaction                                     | 4.2  | 0.8  | 3   | 5   |
| Work engagement                                       | 3.5  | 0.9  | 2   | 5   |
| Organizational commitment                             | 3.8  | 0.7  | 2   | 5   |
| Turnover intention                                    | 1.5  | 0.6  | 1   | 3   |
| Job satisfaction                                      | 3.2  | 0.9  | 2   | 5   |
| Perceived organizational support                      | 3.0  | 0.8  | 2   | 5   |
| Trust in supervisor                                   | 3.1  | 0.7  | 2   | 5   |
| Psychological distance                                | 2.5  | 0.6  | 1   | 4   |
| Perceived social support                              | 3.3  | 0.7  | 2   | 5   |
| Resilience  | 3.6  | 0.8  | 2   | 5   |
| Emotional exhaustion                                  | 1.8  | 0.7  | 1   | 3   |
| Depression  | 1.2  | 0.5  | 1   | 3   |
| Anxiety   | 1.5  | 0.6  | 1   | 3   |
| Life stress   | 2.0  | 0.8  | 1   | 4   |
| Work-life balance                                     | 3.0  | 0.7  | 2   | 4   |
| Organizational justice                                | 3.4  | 0.6  | 2   | 4   |
| Employee voice  | 2.5  | 0.8  | 1   | 4   |
| Organizational citizenship behavior                   | 3.2  | 0.7  | 2   | 4   |
| Organizational identification                         | 3.5  | 0.6  | 2   | 4   |
| Organizational trust                                  | 3.3  | 0.7  | 2   | 4   |
| Organizational commitment (affective)                 | 3.6  | 0.6  | 2   | 4   |
| Organizational commitment (normative)                 | 3.1  | 0.7  | 2   | 4   |
| Organizational commitment (calculative)               | 2.8  | 0.8  | 1   | 4   |
| Organizational identification (cognitive)             | 3.4  | 0.6  | 2   | 4   |
| Organizational identification (emotional)             | 3.2  | 0.7  | 2   | 4   |
| Organizational identification (behavioral)            | 3.0  | 0.8  | 1   | 4   |
| Organizational trust (in organization)                | 3.3  | 0.7  | 2   | 4   |
| Organizational trust (in management)                  | 3.1  | 0.6  | 2   | 4   |
| Organizational trust (in colleagues)                  | 2.9  | 0.7  | 1   | 4   |
| Organizational commitment (affective) (subscale)      | 3.7  | 0.5  | 2   | 4   |
| Organizational commitment (normative) (subscale)      | 3.2  | 0.6  | 2   | 4   |
| Organizational commitment (calculative) (subscale)    | 2.9  | 0.7  | 1   | 4   |
| Organizational identification (cognitive) (subscale)  | 3.5  | 0.5  | 2   | 4   |
| Organizational identification (emotional) (subscale)  | 3.3  | 0.6  | 2   | 4   |
| Organizational identification (behavioral) (subscale) | 3.1  | 0.7  | 2   | 4   |
| Organizational trust (in organization) (subscale)     | 3.4  | 0.6  | 2   | 4   |
| Organizational trust (in management) (subscale)       | 3.2  | 0.5  | 2   | 4   |
| Organizational trust (in colleagues) (subscale)       | 3.0  | 0.6  | 2   | 4   |

Antibodies, soluble proteins comprising for example extracellular domains, and other regulators of CRP1 or B7RP1 that result in prolonged or enhanced T-cell activation can be used to increased the immune response to tumors. Example 20 shows B7RP1-Fc can inhibit tumor cell growth in mice. Similarly, human B7RP1-Fc, or other activators of the B7RP1/CRP1 pathway, may be used to enhance immune responses against human tumors. Anti-tumor activity is generally considered to have a strong cytolytic T-lymphocyte component. In fact, the anti-tumor effects of B7-Fc fusion proteins (Sturmhoefel et al., Cancer Res. 59: 4964-4972, 1999) were mediated by cytolytic CD8+ T-cells. Since CRP1 is also expressed on cytolytic CD8+ T-cells (Example 9), it is probable that the anti-tumor effects demonstrated in Example 20 were due to B7RP1-Fc action on CD8+ cells. The B7RP1/CRP1 pathway can also be manipulated to regulate CTL response in a number of other clinical settings, including allograft transplantation, graft vs. host disease, and autoimmune diseases.

Gene therapy using B7RP1 genes of the invention may be used in cancer immunotherapy. B7RP1 genes introduced into cancer cells can transform them into antigen presenting cells that can be recognized by the T-cells of the immune system when introduced back into an animal. Recognition of the transfected tumor cells by the T-cells results in eradication of both

tumors cells expressing, or not expressing, the B7RP1 gene. This immunotherapy approach may be used for various leukemias, sarcomas, melanomas, adenocarcinomas, breast carcinomas, prostate tumors, lung carcinomas, colon carcinomas and other tumors. This invention encompasses using the B7RP1 gene in a similar manner to enhance T-cell activation in response to variety of tumors.

As described in Example 14, the phenotype of transgenic mice expressing B7RP1 indicates that B7RP1 is important in the control of antibody production. Agonists and antagonists of B7RP1 protein activity may be useful in therapeutic indications that call for the inhibition or enhancement of antibody production.

For instance, many vaccines act by eliciting an effective and specific antibody response. Some vaccines, especially those against intestinal micro-organisms (e.g. Hepatitis A virus, and Salmonellas), elicit a short-lived antibody response. It is desirable to potentiate and prolong this response in order to increase the effectiveness of the vaccine. Therefore, soluble B7RP1 or activating antibodies to CRP1 may serve as a vaccine adjuvant.

Anti-viral responses may also be enhanced by activators or agonists of the B7RP1/CRP1 pathway. The data in Example 20 indicate that cellular immunity is enhanced by B7RP1-Fc. The enhancement of cellular immune functions by B7RP1-Fc, or other activators of the B7RP1/CRP1 pathway, may also be beneficial in eliminating virus-infected cells. In a complementary fashion, B7RP1-Fc has effects on humoral immune functions that may enhance antibody mediated responses as observed in Example 13 that may function to help clear free-virus from the body.

Enhancement of cellular immune functions, would be desirable in treating cancer or viral

infection. Immune response may be enhanced by activation of the B7RP1/CRP1 pathway optionally in conjunction with activation of a separate immune stimulating pathway. A synergistic effect of B7RP1 and B7.2 in a mouse chronic hypersensitivity model is shown in Example 24. Thus, B7RP1 or an agonist of CRP1 may be administered with B7.1 or B7.2 to stimulate immune functions. In another embodiment, B7RP1 or an agonist of CRP1 may be administered with a CD28 agonist or a CTLA4 antagonist to stimulate immune functions. It is also contemplated that B7RP1 or a CRP1 agonist may be used with other immune stimulating molecules, such as T-cell stimulating molecules, in order to enhance cellular immune functions. In one embodiment, a CRP1 agonist is an antibody which binds CRP1 and stimulates or enhances CRP1 activity.

Conversely, there are a number of clinical conditions that would be ameliorated by the inhibition of antibody production. Hypersensitivity is a normally beneficial immune response that is exaggerated or inappropriate, and leads to inflammatory reactions and tissue damage. Hypersensitivity reactions which are antibody-mediated may be particularly susceptible to antagonism by inhibitors of B7RP1 activity. Allergies, hay fever, asthma, and acute edema cause type I hypersensitivity reactions, and these reactions may be suppressed by protein, antibody or small molecule inhibitors of B7RP1 activity.

Diseases that cause antibody-mediated hypersensitivity reactions, including systemic lupus erythematosus, arthritis (rheumatoid arthritis, reactive arthritis, psoriatic arthritis), nephropathies (glomerulo-nephritis, membranous, mesangiocapillary, focal segmental, focal necrotizing, crescentic, proliferative - tubulopathies), skin disorders (pemphigus and pemphigoid, erythema nodosum),

In addition, lymphoproliferative disorders, such as multiple myeloma, Waldenstrom's  
10 macroglobulinemia, and crioglobulinemias, may be inhibited by protein, antibody, or small molecule antagonists of B7RP1.

The B7RP1/CRP1 pathway is involved in regulating IgE production. IgE is an immunoglobulin isotype specifically involved in mediating allergic responses such as asthma, food allergies, hay fever, type 1 hypersensitivity and sinus inflammation. Upon exposure to an allergen, a process involving T-cell and B cell collaboration results in B cell production of IgE specific for the allergen. Allergen-specific IgE released into the circulation by B cells bind to mast cells and basophils through the high affinity IgE receptor (Fc $\epsilon$ RI). Mast cells and basophils to which IgE is bound become sensitized and subsequent exposure to the allergen results in cross-linking of the surface receptors and release of histamines.

30           Example 22 shows that transgenic mice  
expressing a B7RP1 fusion protein have increased levels  
of IgE compared to normal mice. In addition, CRP1  
"knockout" mice show complete inhibition of class  
switching to IgE (see Example 23). These results

| Variable                            | Mean | SD   | Min  | Max  |
|-------------------------------------|------|------|------|------|
| Age                                 | 34.5 | 10.2 | 22   | 55   |
| Gender                              | 0.5  | 0.5  | 0    | 1    |
| Marital status                      | 0.6  | 0.5  | 0    | 1    |
| Education                           | 12.5 | 1.5  | 10   | 15   |
| Income                              | 1500 | 500  | 1000 | 2500 |
| Health status                       | 0.8  | 0.2  | 0    | 1    |
| Smoking status                      | 0.3  | 0.5  | 0    | 1    |
| Alcohol consumption                 | 0.2  | 0.4  | 0    | 1    |
| Exercise frequency                  | 0.5  | 0.5  | 0    | 1    |
| Stress level                        | 0.7  | 0.3  | 0    | 1    |
| Depression score                    | 0.4  | 0.4  | 0    | 1    |
| Life satisfaction                   | 0.6  | 0.3  | 0    | 1    |
| Quality of life                     | 0.7  | 0.2  | 0    | 1    |
| Healthcare utilization              | 0.5  | 0.5  | 0    | 1    |
| Health insurance status             | 0.9  | 0.1  | 0    | 1    |
| Healthcare access                   | 0.8  | 0.2  | 0    | 1    |
| Healthcare cost                     | 1000 | 300  | 500  | 2000 |
| Healthcare quality                  | 0.7  | 0.2  | 0    | 1    |
| Healthcare satisfaction             | 0.6  | 0.3  | 0    | 1    |
| Healthcare utilization frequency    | 0.5  | 0.5  | 0    | 1    |
| Healthcare utilization cost         | 500  | 150  | 200  | 1000 |
| Healthcare utilization quality      | 0.6  | 0.2  | 0    | 1    |
| Healthcare utilization satisfaction | 0.5  | 0.3  | 0    | 1    |
| Healthcare utilization frequency    | 0.5  | 0.5  | 0    | 1    |
| Healthcare utilization cost         | 500  | 150  | 200  | 1000 |
| Healthcare utilization quality      | 0.6  | 0.2  | 0    | 1    |
| Healthcare utilization satisfaction | 0.5  | 0.3  | 0    | 1    |

5  
10  
15  
20  
25  
30  
35

The antagonists of the invention may be used to prevent and/or treat disorders characterized by excessive or inappropriate IgE production. By way of example, such disorders include allergic responses such as asthma, food allergies, hay fever, hypersensitivity, and sinus inflammation.

The invention also provides for the use of a B7RP1 antagonist or a CRP1 antagonist in combination with an IgE antagonist to partially or completely inhibit IgE production and to prevent and/or treat disorders characterized by excessive or inappropriate IgE production. As used herein the term "IgE antagonist" refers to a compound capable of disrupting or blocking the interaction of IgE with its high

affinity receptor  $\text{Fc}_\epsilon\text{RI}$  on cells such that the response to allergen stimulus is attenuated or eliminated. Antagonists include an anti-IgE antibody and fragments thereof, soluble  $\text{Fc}_\epsilon\text{RI}$  receptor and fragments thereof, 5 anti- $\text{Fc}_\epsilon\text{RI}$  antibody and fragments thereof, IgE variants and fragments thereof, IgE binding peptides,  $\text{Fc}_\epsilon\text{RI}$  receptor binding peptides, and small molecules capable of binding to IgE or competing with IgE for binding to  $\text{Fc}_\epsilon\text{RI}$  receptor. B7RP1 antagonists may also be used 10 with in combination with antihistamines, allergen desensitization, reduction in exposure to allergen and the like for treatment of allergic disorders.

In some instances, it may be useful to increase IgE production, such as to prevent and/or 15 treat immune-related disorders in an immune comprised host. In such cases, B7RP1 alone or in conjunction with a CRP1 agonist may be administered in amounts sufficient to increase IgE production.

The invention also provides for the 20 prevention and/or treatment of asthma comprising administering a B7RP1 antagonist or a CRP1 antagonist alone or in conjunction with one or more agents for treating asthma. Examples of such agents include bronchodilators (anti-cholinergic agents,  $\beta_2$  adrenergic 25 receptor agonists, lenkotriene  $\text{D}_4$  antagonists, neurokinin antagonists, potassium channel openers, substance P antagonists, thromboxane  $\text{A}_2$  antagonists, and xanthines), anti-inflammatories (5-lipoxygenase inhibitors, 5-lipoxygenase activating protein 30 inhibitors, phosphodiesterase IV inhibitors, platelet activating factor antagonists, respiratory NSAIDS, steroids, and tyrosine kinase inhibitors), cytokine

inhibitors (CD4, IL-4 and IL-5 inhibitors) and IgE antagonists as set forth above.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

#### Example 1

##### CRP1 cDNA and Amino Acid Sequence

Female C57/ Black 6 mice were sacrificed, and the small intestines were excised, and the Peyer's patches were removed. The small intestine tissue was sliced open and washed to remove mucus and other debris. The epithelial layer, which contains the intestinal intraepithelial cells (iIELs), was released by gentle agitation in RPMI-1640 supplemented with 1 mM dithiothreitol (DTT), for 20 minutes at 37°C. Disassociated cells were passed through a 100  $\mu$  filter, washed in 50 ml of RPMI-1640, mixed to further break up clumps of cells, and then passed through a 40  $\mu$  strainer to obtain single cell populations. These cells were then washed again in a 50 ml volume of RPMI-1640 to ensure the removal of the residual DTT. The tissue was then agitated and washed as before to gather the remaining iIELs. The iIELs were separated from the adipose cells and most epithelial cells on a 3-step Percol gradient, with the iIELs banding at the 40% to 80% interface. These cells were then washed twice with RPMI-1640 to remove traces of Percol, immunostained with CD103 (integrin alpha IEL) antibodies, and separated on a FACs Star cell sorter. These sorted cells were then either used to prepare total RNA directly using Trizol (Gibco BRL, Gaithersburg, MD), or activated overnight on plate-bound activating



antibodies, which crosslink the gamma/delta TCR, alpha/beta TCR, or CD3. The RNA was prepared as above and pooled for use in constructing EST cDNA libraries.

A cDNA clone, designated sm12-00082-a1, contained nucleotide sequence homology to CD28 (Figure 1B). Translation of the sequence and subsequent comparison to known proteins in a public database revealed 19% amino acid identity with murine CD28 (Figure 1B). This low homology was significant because murine CD28 shares only 26% amino acid identity with murine CTLA-4. All of the putative cysteines thought to be critical for intra- and inter-molecular cysteine bonding in the CD28/CTLA-4 family were found to be conserved (amino acid residues 83, 109, and 137; relative to the initiating methionine). In addition, the overall length of the putative open reading frame, and the relative position of the transmembrane domain, were similar to those of both CD28 and CTLA-4. We named the gene CRP1, for CD28-Related Protein-1.

#### Example 2

##### Cloning of Human CRP1 cDNA

The nucleic acid sequence encoding human CRP1 protein is identified by the following procedures. A human cDNA library was prepared from enriched lymphocytes from peripheral human blood from normal human volunteers. The lymphocytes were purified and red blood cells were removed by Lymphocyte Separation Media (ICN Pharmaceuticals, Inc., Costa Mesa, CA). The cells were then activated overnight in media containing 10 ng/ml PMA, 500 ng/ml ionomycin, and plate-bound activating antibodies to CD3. Total RNA was prepared from the activated cells by the Trizol method (Gibco/BRL) and poly A RNA was isolated by Dynal bead purification. cDNA was made from the isolated poly A

RNA and size selected for largest cDNA fragments. The size selected cDNA was then ligated into the plasmid pSPORT (Gibco/BRL). DNA encoding human CRP1 protein is obtained by screening the activated lymphocyte cDNA library by either recombinant bacteriophage plaque, or transformed bacteria colony hybridization protocols (Sambrook et al. *Supra*). The phage or plasmid cDNA library are screened using radioactively-labeled probes derived from the murine CRP1 gene clone as described in Example 1 and Figure 1. The probes are used to screen nylon filters lifted from the plated library. These filters are prehybridized for 4 hr at 42°C in 50% formamide, 5X SSPE, 2X Denhardt's solution, 0.5% SDS, and 100 µg/ml salmon sperm DNA and then hybridized for 24 hr at 42°C in 50% formamide, 5X SSPE, 2X Denhardt's solution, 0.5% SDS, 100 µg/ml salmon sperm DNA, and 5 ng/ml mB7RP1 probe. The blots are washed in 2X SSC, 0.1% SDS for 10 min at RT, 1X SSC, 0.1% SDS for 10 min at 50°C, 0.2X SSC, 0.1% SDS for 10 min at 50°C, then 0.2X SSC for 10 min at 50°C again. Inserts obtained from any human CRP1 clones are sequenced and analyzed as described in Example 1.

### Example 3

#### 25 B7RP1 DNA and Amino Acid Sequence

A cDNA clone, designated sm11-00003-g5, contained nucleotide sequence homology to B7.1 (CD80) and B7.2 (CD86). Translation of the sequence (Figure 2A) and subsequent comparison to known proteins in a public database revealed 20% amino acid identity with murine B7.1 (Figure 2B). This low homology was significant because murine B7.1 shares only 24% amino acid identity with murine B7.2. Despite this low homology, critical cysteine residues are conserved

between the open reading frame of this clone and murine B7.1 and B7.2 at residues 62, 138, 185, and 242 (relative to the initiating methionine, Figure 2B). The approximate mature protein length and the location of the transmembrane region relative to the carboxy terminus are also similar in the putative ORF of this clone, as compared to B7.1 and B7.2. We named the gene B7RP1, for B7-Related Protein-1.

10

Example 4

## Cloning of Human B7RP1 cDNA

A Genbank blast homology search (GCG, University of Wisconsin) using murine B7RP1 sequence (see Figure 2) retrieved a clone (AB014553) containing a 4358 bp sequence with 1679 bp of ORF. PCR cloning primers were designed according to this sequence. A DNA fragment of 1313 bp was obtained by 5' and 3' RACE using Human Lymph Node Marathon-Ready™ cDNA (Clontech, Palo Alto, CA) according to the manufacturer's recommended procedures. Primers used for full length human B7RP1:

|            |                                       |
|------------|---------------------------------------|
| 2083-75    | ACC ATG CGG CTG GGC AGT CCT GGA       |
| 25         | (SEQ ID NO: 25)                       |
| 2083-76    | TGG TGA CCT ACC ACA TCC CAC AG        |
|            | (SEQ ID NO: 26)                       |
| 2083-77    | TCC GAT GTC ATT TCC TGT CTG GC        |
|            | (SEQ ID NO: 27)                       |
| 30 2083-78 | GCT CTG TCT CCG GAC TCA CAG CCC       |
|            | (SEQ ID NO: 28)                       |
| 2113-29    | GTG GCA GCA AAC TTC AGC GTG CCC GTC G |
|            | (SEQ ID NO: 29)                       |
| 2113-30    | CCC AAC GTG TAC TGG ATC AAT AAG ACG G |
| 35         | (SEQ ID NO: 30)                       |
| 2113-31    | GCG TGC TGA GGA TCG CAC GGA CCC CCA G |

(SEQ ID NO: 31)

Primers 2083-75 and 2083-76 were used to amplify the 5' end of the gene using RACE protocols. Primers 2083-77, 2083-78, 2113-29, 2113-30, and 2113-31, were used to amplify the 3' end of the gene using RACE protocols.

The resulting nucleotide sequence contained an ORF of 288 amino acid residues beginning at the methionine. The predicted mature human B7RP1 amino acid sequence was then compared to the mature mouse B7RP1 amino acid sequence (Figure 3B) and found to share 48% amino acid identity. This homology is significant because the homology between species is low with the CD80 (B7.1) gene, in fact, the mouse and human CD80 share only 41% amino acid identity. Importantly, the human B7RP1 protein conserve critical cysteine residues necessary for Ig loop structures (amino acid residues 16, 92, 138, 194, and 195, relative to the mature protein, Figure 3B). In addition, the overall length and position of the transmembrane domain are consistent with a human B7RP1 homolog.

#### Example 5

##### Expression of B7RP1 RNA

RNA in situ hybridization using RNA probes to the B7RP1 gene. Adult mouse tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5  $\mu$ m. Prior to in situ hybridization, tissues were permeabilized with 0.2M HCL, followed by digestion with Proteinase K, and acetylation with triethanolamine and acetic anhydride. Sections were hybridized overnight at 55°C with a 969 base <sup>33</sup>P-labeled riboprobe corresponding to nucleotides 1 to 969 of the mouse B7RP1 sequence. Excess probe was removed by RNase

digestion, followed by a series of washes in buffer with decreasing salt concentrations, and then a high stringency wash in 0.1X SSC at 55°C. Slides were dipped in Kodak NTB2 emulsion, exposed at 4°C for 2-3 weeks, developed, and counterstained with hematoxylin and eosin. Sections were examined with darkfield and transmitted light illumination to allow simultaneous evaluation of the tissue morphology and the hybridization signal.

The analysis of the B7RP1 RNA by in situ hybridization showed that the B7RP1 RNA was highly expressed in areas of lymphoid maturation and lymphocyte activation. B7RP1 RNA was expressed in the lymphoid tissues of the thymus, Peyer's patches of the intestine, spleen, and lymph nodes. Expression within these lymphoid tissues demonstrated that the B7RP1 RNA was generally expressed in the areas of B-cell and other APC involvement. These regions include the medulla area of the thymus, the primary follicles of the lymph nodes, and the follicular and dome regions of the Peyer's patches. The expression of B7RP1 RNA is highly specific to the regions of APC involvement in lymphoid tissues.

The analysis of several non-lymphoid tissues also revealed B7RP1 expression in regions of APC involvement. In the lung, B7RP1 expression was found in the submucosal regions, consistent with a function in antigen processing. In the small intestine, B7RP1 RNA was found in the lamina propria. Notably, we found a section of damaged liver, which showed lymphocyte infiltration that overlapped with the expression of B7RP1 RNA. This coincidence of B7RP1 expression with lymphocyte accumulation in response to tissue damage strongly indicates that B7RP1 is involved in lymphocyte activation.

Example 6

## Expression of CRP1 RNA

RNA in situ hybridization using RNA probes to  
5 the CRP1 gene. Mouse tissues were prepared as in  
Example 5. Tissue permeabilization, probe  
hybridization, slide treatment, and tissue staining  
were as described in Example 5. Sections were  
hybridized overnight at 55°C with a 603 base <sup>33</sup>P-  
10 labeled riboprobe corresponding to nucleotides 1 to 603  
of the mouse CRP1 sequence. Sections were examined  
with darkfield and transmitted light illumination to  
allow simultaneous evaluation of the tissue morphology  
and the hybridization signal.

15 Lymph nodes from normal mice or a mouse  
treated with oxazolone were sectioned and analyzed for  
CRP1 RNA expression. The sensitized mouse lymph node  
showed greater expression of CRP1 RNA than the normal  
mouse lymph node. The expression of CRP1 was in the  
20 paracortex, a region of T-cell activity. Therefore,  
the expression of CRP1 RNA is consistent with that of  
T-lymphocyte expression and is up-regulated upon T-cell  
activation.

25

Example 7Expression and Purification of  
CRP1-Fc and B7RP1-Fc Fusion Proteins

30 To construct the DNA expression vector for  
the CRP1- Fc fusion protein, the coding sequence for  
the first amino terminal 147 amino acids of the CRP1  
was fused, inframe, to the coding sequence for the  
carboxy terminal 235 amino acids of the human Fc gene  
35 (isotype IgG1) and ligated within the polylinker

sequence of pcDNA3 (pcDNA3/CRP1-Fc). To construct the DNA expression vector for the B7RP1- Fc fusion protein, the coding sequence for the first amino terminal 269 amino acids of the B7RP1 was fused, inframe, to the coding sequence for the carboxy terminal 235 amino acids of the human Fc gene (isotype IgG1) and ligated within the polylinker sequence of pcDNA3 (pcDNA3/B7RP1-Fc). The coding sequences of both CRP1 and B7RP1 contained sequences from the N-terminus of each protein up to, but not including, the putative transmembrane region of each protein. 293T-cells were transfected with either pcDNA3/CRP1-Fc or pcDNA3/B7RP1-Fc using the FuGene 6 transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN). After four days, the conditioned media were collected and the Fc fusion proteins were purified by batch chromatography using Protein A Sepharose (Pharmacia). Fc fusion proteins bound to the column were eluted with three column volumes of Immunopure Gentle Elution Buffer (Pierce), and then were dialyzed against 150 volumes of 20 mM HEPES, 100 mM NaCl, pH 7.5. The dialyzed protein was concentrated using Macrosep centrifugal concentrates, 30 kD MWCO (Pall Filtron), and the protein concentrations were calculated using extinction coefficients derived from the amino acid sequence of each protein. Expression of CRP1-Fc fusion protein is shown in Figure 4A, expression of B7CPR1-Fc fusion protein is shown in Figure 4B.

#### Example 8

##### Identification of CRP1 and B7RP1 as a Receptor-ligand Pair

In order to determine whether the novel proteins were part of the same costimulatory pathway as

that containing CD28, CTLA-4, B7.1, and B7.2, we utilized a cell surface display assay. This assay uses ACAS (Adherent Cell Analysis and Sorting) analysis to analyze whether membrane-bound proteins expressed in  
5 cells interact with various Fc fusion proteins. Cells expressing membrane-bound proteins, indicated on the left side of Figure 5, were incubated with Fc fusions proteins, indicated at the top of the figure.

Cos-7 cells, grown in DMEM media with 10%  
10 FBS, were plated at 500,000 cells/well in a 24-well plate. Cells were transfected using the FuGene 6 reagent (Roche Molecular Biochemicals, Indianapolis, IN). For each transfection, 3  $\mu$ l of FuGene 6 reagent was added to 47  $\mu$ l of serum free DMEM media. After a  
15 10 min incubation at room temperature, the mix was added to 0.25  $\mu$ g of plasmid dropwise and then was incubated for 15 minutes. The above mix was then added to the cells with 0.5 ml of DMEM with 10% FBS. The cells were incubated at 37°C in a 5% CO<sub>2</sub> atmosphere.  
20 As a control, CHO D- cells, stably transfected with an expression plasmid containing the cDNA for human CD28, were also plated at 500,000 cells/well in a 24-well plate.

After 48 hr, the medium with transfection  
25 reagent was removed and the cells were washed twice with RPMI plus 5% FBS. 10 to 20 ng of purified Fc fusion proteins in 1 ml of media were added to the cells, which were incubated for 30 min on ice. The cells were washed three times with RPMI plus 5% FBS and  
30 then were incubated with 2  $\mu$ l of FITC-conjugated anti-human Fc antibody (1 mg/ml) for another 30 min on ice. After three successive washes with RPMI, the cells were covered with 250  $\mu$ l of RPMI media without phenol red for ACAS analysis.



ACAS analysis of the cells that bound the various Fc fusion proteins demonstrated that the B7RP1 protein bound CRP1, but not the proteins in the known costimulatory pathway, CD28 or CTLA-4. Conversely, CRP1 interacted with B7RP1, but not B7.2, a component in the known pathway. (See Figure 5). These results strongly indicate that CRP1 and B7RP1 represent a novel receptor-ligand pair, analogous to CD28 and B7.2. However, since CRP1 and B7RP1 do not interact with B7.2, CTLA-4, or CD28, they are separate and independent of the known costimulatory pathway.

#### Example 9

##### 15 Identification of Cells Expressing B7RP1 Receptors

The B7RP1-Fc fusion protein was utilized to detect cells that expressed receptors to B7RP1, presumably including the CRP1 protein (see Example 6), by FACS analysis. Spleens were removed from female C57/Black 6 mice, ground on 100 micron mesh filters to release the lymphocytes, passed through 70 micron filters, and then washed in 50 ml of RPMI-1640. They were pelleted at 1500 rpm, resuspended in fresh RPMI, mixed to break up the clumping cells, and passed through a 40 micron filter. T-cells to be activated were seeded into 6 well plates in RPMI-1640, 5% FBS, 1XPSG, PMA, ionomycin, and incubated at 37°C, 5% CO2 overnight. T-cell activation was checked by visual confirmation after 12 hr.

Activated spleen cells for immunostaining were washed in PBS, 0.5% BSA (Path-ocyte 4, ICN Pharmaceuticals) wash buffer, resuspended, and then aliquoted in 100  $\mu$ l volumes. 15  $\mu$ g/ml of either the CRP1-Fc fusion protein or the B7RP1-Fc fusion protein was added (1.5  $\mu$ g/sample) as appropriate, and then the

mixtures were incubated on ice for 30 min with occasional mixing. The cells were washed twice in 5.0 ml of wash buffer. Binding of the fusion proteins was visualized with 2  $\mu$ g of goat-anti-human (GaHuFc-FITC) conjugated secondary antibody in a 100  $\mu$ l volume for cell staining. Cell marker antibodies conjugated with PE were added with the GaHUFc-FITC, as well as control isotype-PE conjugated antibody controls where indicated (rat isotype). The samples were incubated on ice and washed as before. Visualization was done by FACScan analysis with gating on the lymphocyte populations. Double staining with CD4+ antibodies and the B7RP1-Fc fusion protein indicated that the cells expressed both the CD4 marker and the receptor to B7RP1, presumably CRP1 (Figure 6). Similarly, double staining with CD8+ antibodies and the B7RP1-Fc fusion protein demonstrated that cells expressed both CD8 and B7RP1 receptors (Figure 6). We could not reliably detect such double staining cells in inactivated splenocyte preparations. Since CD4 and CD8 are T-lymphocyte markers, we can postulate that CRP1 is expressed on activated CD4+ and CD8+ T-cells. These data are consistent with the increased expression of CRP1 RNA in the T-cell regions of lymph nodes from sensitized mice as compared to normal mice (Example 6).

#### Example 10

##### Identification of Cells Expressing CRP1 Ligands

The CRP1-Fc fusion protein was utilized to detect cells that expressed ligands to CRP1, presumably including the B7RP1 protein (see Example 8), by FACS analysis (Figure 7). Splenocytes were prepared as in Example 8, except the 12 hr T-cell activation step was omitted and the cells were directly analyzed. Splenocytes were double stained with CD45R (B220)

marker antibodies and the CRP1-Fc fusion protein. Cells were detected that expressed both the CD45R B-cell marker and the putative ligands for CRP1, presumably including B7RP1 (Example 8). Therefore, we  
5 conclude that B7RP1 is expressed on B-cells, a type of antigen-presenting cell. These data are consistent with the expression of B7RP1 RNA in B-cell regions of various lymphoid tissues (Example 5).

FACS analysis of the expression of B7RP1 on  
10 peritoneal macrophages (Figure 8). Peritoneal cells were collected by local lavage from a normal mouse and washed before being incubated with the CRP1-Fc fusion protein or the Fc protein as a control or with the F4/80 monoclonal antibody (which detects an antigen  
15 specific for macrophages) or an irrelevant, isotype-matched control monoclonal antibody. Cells were then washed again and incubated with goat-anti-human Fc-FITC conjugated antibody. After further washing, cells were assessed in a FACS analyzer for their light scattering  
20 and fluorescence staining properties. Peritoneal cells were first distinguished in subsets on the ground of their light scattering properties (Figure 8A). Macrophages were identified in region 5 (R5) because of their ability to strongly scatter light forward (FSC)  
25 and sideways (SSC) and because of their positive staining for the F4/80 antigen, a marker for macrophages (Figure 8B). Macrophages in region 6 (R6) were singled out on the basis of their less intense staining for the F4/80 antigen and found to be stained  
30 by the CRP1-Fc fusion protein (Figure 8C). These data indicate that ligands for CRP1, possibly including B7RP1, are expressed on macrophages, a professional antigen presenting cell. This is consistent with CRP1 and B7RP1 function in T-lymphocyte activation.

35

Example 11

In vitro Inhibitory Activity of the B7RP1-Fc Fusion  
Protein on ConA-Stimulated T-Lymphocytes

Mouse splenocytes were prepared as in Example  
5 8 and enriched for T-lymphocytes by negative selection  
(R and D Systems, Inc., Minneapolis, MN)). 200,000  
splenocytes were used in T-cell proliferation assays in  
a 96-well round-bottom plate. Cells were incubated for  
1 hr with media (no adds), CRP1-Fc, B7RP1-Fc, or B7.2-  
10 Fc, fusion proteins as indicated Figure 9. Media (no  
adds), or Con A at various concentrations were added as  
indicated in at the bottom of Figure 9. The cells were  
then incubated at 37°C and 5% CO<sub>2</sub>. After 42 hr, cells  
were pulsed with 3H-thymidine for 6 hr, harvested and  
15 incorporated radioactivity determined. Average CPM and  
standard deviation from triplicate samples are  
represented in Figure 9.

The Fc fusion proteins did not demonstrate  
significant T-cell stimulatory or inhibitory activity  
20 by themselves, however, in the presence of 1 µg/ml and  
3 µg/ml Con A, both the B7RP1-Fc and the known B7.2-Fc  
fusion proteins showed significant inhibitory activity  
(Figure 9). At high concentrations (10 µg/ml), Con A  
stimulation results in cell death, presumably through  
25 over-activation of the T-cells. Addition of either  
B7RP1-Fc or B7.2-Fc, significantly protected the cells  
from the detrimental effects of high concentrations of  
Con A. In both inhibitory and protective functions,  
the effect by B7RP1-Fc protein was greater than B7.2-Fc  
30 protein on the Con A stimulated cells. These data  
indicate that the B7RP1 protein functions to regulate  
T-cell proliferation.

Example 12

35        Systemic delivery of B7RP1-Fc fusion protein in  
transgenic mice

09724420 112800

The B7RP1-Fc fusion protein described in Example 7 was subcloned into an ApoE-liver specific expression vector (Simonet et al. J. Clin. Invest. 94, 1310-1319 (1994) and PCT Application No. US94/11675). The coding region was excised from pCEP4/B7RP1-Fc using the restriction enzymes, Spe I and Not I, and the fragment subcloned into the same sites in the previously mentioned ApoE-liver specific expression vector. The resultant plasmid, HE-B7RP1-Fc, was sequenced through it's protein coding region, and sequences flanking the coding region, to ensure it was mutation free.

The plasmid was amplified and purified through two rounds of CsCl density gradient centrifugation. The purified plasmid DNA was digested with the restriction enzymes, Cla I and Ase I, and the 1.5 kb transgene insert was purified by agarose gel electrophoresis. The purified fragment was diluted to a stock injection solution of 1  $\mu$ g/ml in 5 mM Tris, pH 7.4, and 0.2 mM EDTA. Single-cell embryos from BDF1 X BDF1-bred mice were injected essentially as described (Brinster et al., Proc. Natl. Acad. Sci. USA 82, 4338 (1985)), except that injection needles were beveled and siliconized before use. Embryos were cultured overnight in a CO2 incubator and 15 to 20 2-cell embryos were transferred to the oviducts of pseudopregnant CD1 female mice.

Following term pregnancy, 56 offspring were obtained from implantation on the microinjected embryos. The offspring were screened by PCR amplification of the integrated transgene in genomic DNA samples. The target region for amplification was a 369 bp region of the human Apo E intron which was included in the expression vector. The oligos used for PCR amplification were:

5'-GCC TCT AGA AAG AGC TGG GAC-3' (SEQ ID NO: 32)

5'-CGC CGT GTT CCA TTT ATG AGC-3' (SEQ ID NO: 33)

5           The conditions for the PCR were: 94°C for 2 min, 1 cycle; 94°C for 1 min, 63°C for 20 sec, and 72°C for 30 sec, 30 cycles. Of the 56 original offspring, 7 were identified as PCR positive transgenic founder mice.

10           At 12 weeks of age, nine transgenic founders (mouse #1, 2, 4, 6, 8, 30, 32, 33, 40) and five controls (mouse #5, 9, 10, 25, 28) were sacrificed for necropsy and pathological analysis. Total cellular RNA was isolated from the livers of the founder animals and  
15 negative control littermates as described (McDonald et al. Meth. Enzymol. 152, 219 (1987)). Northern blot analysis was performed on these samples to assess the level of transgene expression. Approximately 10 µg of total RNA from each animal was resolved by agarose  
20 electrophoresis denaturing gels (Ogden et al. Meth. Enzymol. 152, 61 (1987)), then transferred to HYBOND-N nylon membrane (Amersham), and probed with <sup>32</sup>P dCTP-labeled mB7RP1-Fc insert DNA. Hybridization was performed for 1 hr at 63°C in ExpressHyb Solution  
25 (Clonetech) and 2-4 X 10<sup>6</sup> CPM of labeled probe/ml hybridization buffer. Following hybridization, blots were washed twice in 2X SSC, 0.1% SDS at room temperature for 5 min each, and then twice in 0.1X SSC, 0.1% SDS at 55°C for 15-20 min each. Expression of the  
30 transgene in founder and control littermates was determined following autoradiography.

          Northern blot analyses indicated that seven of the transgenic founders expressed detectable levels of the transgene RNA (mouse #1, 2, 6, 8, 32, 33, and  
35 40). The negative control mice and three founders (#4,

008211 02482/60

30, and 31) did not express detectable levels of RNA. Since the B7RP1-Fc fusion protein was determined to be secreted from mammalian cells in culture (Figure 4B and Example 7), expression of the transgene mRNA should be indicative of the level of systemically delivered gene product.

### Example 13

#### Biological activity of B7RP1-Fc fusion protein

10

Seven of the transgenic mice (mouse #1, 2, 6, 8, 32, 33, and 40) and five control littermates (#5, 9, 10, 25, and 28) were sacrificed for necropsy and pathological analysis using the following procedures:

15 Prior to euthanasia, all animals had their identification numbers verified, then were weighed, anesthetized and blood drawn. The blood was saved as both serum and whole blood for a complete serum chemistry and hematology panel. Radiography was

20 performed just after terminal anesthesia by lethal CO<sub>2</sub> inhalation, and prior to gross dissection. Tissues were then removed and fixed in 10% buffered Zn-formalin for histological examination. The tissues collected included the liver, spleen, pancreas, stomach,

25 duodenum, ileum, Peyer's patches, colon, kidney, reproductive organs, skin, mammary glands, bone, brain, heart, lung, thymus, trachea, esophagus, thyroid/parathyroid glands, jejunum, cecum, rectum, adrenal glands, white and brown fat, sciatic nerve,

30 bone marrow, urinary bladder, and skeletal muscle. Prior to fixation, the whole organ weights were determined for the liver, heart, stomach, kidney, adrenals, spleen, and thymus. After fixation, the tissues were processed into paraffin blocks, and 3  $\mu$ m

35 sections were obtained.

Immunohistochemistry for the B-lymphocyte marker, B220, and the T-lymphocyte marker, CD3, was performed. To detect B220 or CD3 expression, formalin fixed, paraffin embedded, 4  $\mu$ m sections were  
5 deparaffinized and hydrated to deionized water. The sections were quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw, Pittsburgh, PA), and incubated in rat monoclonal antibody to B220 (Pharmingen, San Diego, CA) or rabbit polyclonal  
10 antibody to CD3 (Dako, Carpinteria, CA). The antibodies were detected by biotinylated rabbit anti-rat or goat anti-rabbit immunoglobulins, peroxidase conjugated streptavidin, (BioGenex, San Ramon, CA) with DAB as chromagen (Biotek, Santa Barbara, CA). Sections  
15 were counterstained with hemaoxylin.

In this study, normal clinical signs were reported during the in-life phase of the study. The whole body radiographs of the transgenic mice were comparable to those of the control mice. The overall  
20 hematologic parameters of the transgenic mice were comparable to those of the negative control group, although sporadic changes in individual mice were present: transgenic #8 and #40 had increased serum globulin levels (hyperglobulinemia) and #32 and #33 had  
25 globulin levels in the high normal range accompanied by albumin levels in the low normal range, which is a pattern commonly seen with chronic antigenic stimulation of the immune system. Organ weights of the other transgenic mice were not significantly different  
30 from those of the control group.

The following histopathological changes were present in the transgenic mice: The mesenteric lymph nodes of the transgenic B7RP1-Fc mice were moderately to markedly enlarged when compared to the control mice  
35 (Fig. 10A-10D; Fig. 11A-11E). The cortex had prominent



follicular hyperplasia seen as enlarged secondary follicles (Fig. 10B-11B) with large germinal centers containing mostly B220+ B cells (Fig. 11D) and a few scattered CD3+ T cells (Fig. 11F). The paracortical (CD3+ T-cell) area was also moderately enlarged (Fig. 11B-11F) and the medullary sinuses had slightly increased numbers of fleshy macrophages (sinus histiocytosis). The most conspicuous change in the nodes was present in the medullary cords, which were mildly to markedly expanded by large numbers of well-differentiated plasma cells in the B7RP1-Fc transgenic mice (Fig. 10D). In transgenic mouse #40, small numbers of scattered Russell bodies (i.e. plasma cells with prominent, large, round, intracytoplasmic vesicles containing immunoglobulins) were also found in the medullary cords (Fig. 10D). Interestingly, the other internal and peripheral lymph nodes (e.g. cervical, inguinal) had similar morphologic features of reactive lymphoid hyperplasia suggestive of a systemic response. These findings are consistent with a chronic, ongoing immune stimulation with enhancement of the humoral immune reaction, which leads to B cell proliferation and terminal differentiation into plasma cells.

The spleen of B7RP1-Fc transgenic mice had variably enlarged white pulp areas with moderate reactive lymphoid hyperplasia involving particularly the B-cell secondary follicles with prominent germinal centers and periarteriolar T-cell sheaths when compared to the control mice (Fig. 10E-10F). Another conspicuous finding in B7RP1-Fc transgenic mice was minimal to mild plasmacytosis in the marginal zone surrounding the white pulp areas and in the adjacent red pulp. Transgenic mouse #6 had a few scattered Russell bodies (Fig. 10F, inset). The red pulp had mild to moderate extramedullary hematopoiesis, which

was comparable to that seen in the control mice (Fig. 10E).

The small intestinal Peyer's patches were mildly to markedly enlarged in the B7RP1-Fc transgenic mice over those of the control mice (Fig. 10G) and had very large follicles with prominent germinal centers, particularly in transgenic mouse #40 and #32 (Fig. 10H). In addition, there was a minimal (in #32) to mild (in #8 and #33) increase in the numbers of lymphocytes and plasma cells (admixed with a mild eosinophil infiltrate in the ileum of mouse #32) in the thickened lamina propria layer of the mucosa, which was present in the small intestine, but more prominent in the colon of the transgenic mice. The large intestinal lymphoid aggregates (GALT) were also slightly more prominent in some B7RP1-Fc transgenic mice (particularly mouse #8 and #2) than in the control group.

Generally, the other tissues examined, including the thymus, bone marrow, liver, lung, heart, pancreas, kidneys, adrenal gland, thyroid, parathyroid, trachea, reproductive organs, urinary bladder, mammary gland, skin, skeletal muscle, peripheral nerve, brain, esophagus, stomach, small and large intestine, bone (femur/tibia), stifle joint, white and brown fat appeared normal and comparable to the background changes detected in the control mice.

The data from this study demonstrate that overexpression of the B7-related protein Fc chimera (B7RP1-Fc) in transgenic mice induces a phenotype characterized by prominent reactive lymphoid hyperplasia detected in the spleen, peripheral and internal lymph nodes, and gut-associated lymphoid tissue, as follicular hyperplasia, expansion of T-cell areas and conspicuous plasmacytosis accompanied by hyperglobulinemia in some animals. The plasmacytosis

is accompanied by higher levels of circulating IgG (mean  $\pm$  SD =  $597 \pm 298$  mg/ml in transgenic mice vs.  $209 \pm 80$  mg/ml in control littermates,  $n = 7$ ,  $P < 0.05$ ,  $t$  test), in particular IgG2a ( $217 \pm 100$  mg/ml vs.  $75 \pm 29$  mg/ml,  $n = 7$ ,  $P < 0.01$ ,  $t$  test). The induction of IgG2a is normally associated with a Th1 cytokines such as IFN- $\gamma$ . Thus, B7RP1 induces B- and T-cell proliferation and stimulates B-cells to differentiate into plasma cells and to produce immunoglobulin.

These changes are consistent with a persistent systemic immune response with hyperstimulation of the humoral arm of the immune system which results in B cell stimulation, proliferation, and differentiation to antibody-producing plasma cells throughout the lymphoid organs examined.

We conclude from the marked lymphoid hyperplasia demonstrated in the B7RP1-Fc transgenic mice that B7RP1 protein has significant *in vivo* biological activity, related to immune system stimulation.

#### Example 14

##### Cloning of human B7RP1

Normal human circulating peripheral lymphocytes were separated from red blood cells using Lymphocyte Separation Medium (ICN Pharmaceuticals). The T-cells were then activated with  $10 \mu\text{g/ml}$  plate bound anti-CD3 antibody (Immunotech, Westbrook, ME),  $10 \text{ ng/ml}$  PMA, and  $500 \text{ ng/ml}$  ionomycin overnight (16 hours) at  $37^\circ\text{C}$  and  $5\% \text{ CO}_2$ . Total RNA was then prepared from the cells using TRIzol reagent (Gibco BRL). The cells were pelleted by centrifugation and the cell pellet was resuspended in  $1 \text{ ml}$  TRIzol reagent for each  $5 \times 10^6$  cells and incubated at room temperature for 5 min.  $0.2$

ml chloroform per 1 ml original TRIzol reagent was then added. The tubes were shaken vigorously by hand for 15 seconds and incubated for 3 minutes at RT and centrifuged at 13,000rpm for 15 min at 4°C. Following  
5 centrifugation, the clear upper aqueous phase which contains the RNA was collected and the sample RNA was precipitated by the addition of isopropyl alcohol. The solution was then incubated at RT for 10 min, the RNA pelleted, washed with 75% ethanol, and then centrifuged  
10 at 15,000 rpm for 5 min at 4°C. The pellet was air dried, resuspend in RNase-free water, then aliquoted, and stored at -80°C until later use.

The library was constructed using the SuperScript Plasmid System for cDNA Synthesis and  
15 Plasmid Cloning (Gibco BRL). Briefly, cDNA inserts with an average size of 2 kb, were ligated into the pSport vector at Sal1/ Not1 cloning site. The ligated plasmids were electroporated into Electromax transformation competent *E.coli* (Gibco BRL), titered  
20 and plated at fifteen thousand colonies per LB plate (ampicillin 100 µg/ml). 300,000 colonies were lifted onto colony/plaque screen hybridization transfer membranes (NEN Life Sciences), denatured in 0.5 N NaOH, 1.5 M NaCl for 5 minutes, then neutralized successively  
25 for 5 minutes each in the following buffers, 1 M Tris HCl pH 8.0, 0.5 M Tris HCl pH 8.0 and 1.5 M NaCl and 2X SSC. The filters were then crosslinked by ultraviolet irradiation and baked for 30 min at 80°C in a vacuum oven. The filters were pre-washed extensively  
30 in 2X SSC at 42°C to remove debris, then prehybridized at 42°C in 50% formamide, 5X SSPE, 5X Denhardt's solution, 0.5% SDS, 100 µg/ml salmon sperm DNA, for 2 hours.

The human lymphocyte cDNA library was  
35 screened with an 895 bp DNA fragment having nucleotides

1-711 as shown in Figure 3A, 167 bps immediately 5' to the initiator methionine codon in Figure 3A and 17 bps immediately 3' to position 711 in Figure 3A. This upstream 5' sequence of 167 base pairs was obtained by  
5 5' RACE of the HuB7RP1 cDNA (Example 4) and was released from a TOPO TA vector (Invitrogen, Carlsbad, CA) at the Eco RI restriction enzyme cleavage site. This insert was twice purified on a 0.8% agarose TAE gel. A DNA gel purification kit (Qiagen) was used to  
10 isolate the DNA insert from the agarose.

125 ng of the DNA fragment was labeled with <sup>32</sup>P dCTP (Amersham) following the Redi-Prime 2 (Amersham) random prime labeling system protocol. The colony lift filters were then allowed to hybridize with the probe  
15 at 42°C in the following buffer overnight at 42°C; 50% formamide, 5X SSPE, 2X Denhardt's solution, 0.5% SDS, 100 mg/ml ssDNA. The specific activity of the probe was  $2.38 \times 10^9$  cpm/ $\mu$ g DNA, in approximately 2 ng labeled probe per ml hybridization buffer. The probe  
20 was removed and saved for the next round of screening. The filters were then washed in 2X SSC, 0.1% SDS RT for 15 min, followed by 1X SSC, 0.1% SDS at 55°C for 15 min, and 1X SSC, 0.1% SDS at 60°C for 10 min. The filters were wrapped in plastic and exposed to  
25 autoradiography film overnight at -80°C with 2 enhancing screens. Three independent positive clones were identified. Exposures were aligned to the bacterial plates and the positive clones scraped, diluted and replated on LB plates with ampicillin  
30 100  $\mu$ g/ml, grown overnight as before and the colonies were lifted, prepared, and probed as described previously. Three independent clone colonies were isolated, the DNA was isolated, and DNA sequenced for each clone in triplicate.

35 The full-length of the human B7RP1 protein is 302 amino acids. The polypeptide length and relative

10

## Cloning of human CRP1

20

Using the above primers, a 151 bp DNA  
25 fragment of the murine CRP1 was PCR amplified using the  
murine CRP1 plasmid described in Figure 1 and Example 1  
as template. 125 ng of the DNA was labeled with  $^{32}\text{P}$   
dCTP (Amersham) following the Redi-Prime 2 (Amersham)  
random prime labeling system protocol. The colony lift  
30 filters from human peripheral blood libraries described  
in Example 15 were then allowed to hybridize with the  
probe in the following hybridization buffer overnight  
(15 hr) at  $41^{\circ}\text{C}$ , 50% formamide, 5X SSPE, 2X Denhardt's  
solution, 0.5% SDS, 100  $\mu\text{g/ml}$  ssDNA. The specific  
35 activity of the probe was  $3.52 \times 10^9$  cpm/ $\mu\text{g}$  DNA, 1.5 ng  
labeled probe/ml hybridization buffer. The probe was

pulled off and saved for the next round of screening. The filters were then washed in 2X SSC, 0.1% SDS at RT for 10 min, followed by 1X SSC, 0.1% SDS at 37°C for 7 minutes, 40°C for 7 minutes, 44°C for 7 minutes, then  
5 50°C for 7 minutes, continually monitoring the rate at which the filters were releasing the labeled probe. The filters were wrapped in plastic and exposed to film overnight at -80°C with 2 enhancing screens. This method revealed 9 possible independent positive clones.  
10 Exposures were aligned to the bacterial plates and the positive clones scraped, deposited into 200  $\mu$ l SOC, 2 serial dilutions of 1:10 were performed and 70  $\mu$ l from the second dilution was replated on LB plates containing ampicillin at 100  $\mu$ g/ml and grown overnight  
15 as before. The colonies were lifted, prepared and probed as before. Eight independent clones were isolated and DNA prepared by the Qiagen miniprep method.

A cDNA clone containing an open reading frame  
20 of 199 amino acids was obtained (Figure 13A). This cDNA clone contained nucleotide and amino acid homologies to the murine CRP1 clone described in Example 1 and Figure 1. The nucleotides corresponding to the open reading frame of this human clone was 77%  
25 identical to the murine CRP1 gene. Translation of the human sequence and subsequent comparison with the murine CRP1 protein revealed 69% amino acid identity with the murine protein (Figure 13B). In addition, the motif between amino acids 114 to 119, "FDPPPF", was  
30 conserved between the murine and human CRP1 genes. This motif corresponds to the "MYPPPY" motif in murine and human CD28 that is essential for B7 protein interaction. Furthermore, the cysteines at amino acid positions 42, 109, and 141 are also conserved. These  
35 cysteines correspond to cysteines in CD28 and CTLA-4 at

are involved in Ig loop formation and intermolecular disulfide dimerization. The close similarity with murine CRP1, and structural similarities with the CD28 homology family, indicate that this is the human CRP1 homolog.

#### Example 16

CRP1 is expressed on resting memory T-lymphocytes

10

In order to study CRP1 expression on memory T-cells, splenic T-cells were collected from 6-7 month old mice. These cells were double-stained using B7RP1-Fc labeled by an FITC-conjugated anti-human Fc antibody and a PE-conjugated antibody to either CD44, CD45RB, or CD69. Staining with the B7RP1-Fc fusion protein detects expression of CRP1 protein on these T-cells. Older mice show more CRP1+ splenic T-cells than younger mice. Interestingly, a conspicuous number of these cells are CD44 high (Fig 14a) and CD45RB low (Fig 14b), a profile typical of memory T-cells. These CRP1+ memory T-cells are in a resting state, since they do not express the activation marker CD69 (Fig. 14c). The expression of CRP1 on memory T-cells indicates that CRP1 has costimulatory functions on memory T-cells.

#### Example 17

*In vitro* T-cell costimulation  
inhibited by antibodies to B7RP1.

30

To determine if the B7RP1 protein has functional relevance to T-cells, we incubated CD3+ T-cells with the B7RP1-Fc fusion protein and an anti-CD3 antibody in an *in vitro* proliferation assay. Rabbit anti-mouse B7RP1 polyclonal antibodies or rat anti-mouse B7RP1 monoclonal antibodies were then used to

35



specifically inhibit B7RP1-Fc costimulated proliferation *in vitro*.

B7RP1 rabbit polyclonal antiserum preparation

5           Three New Zealand white rabbits (5-8 lbs. initial weight) were injected IM with murine B7RP1 protein. Each rabbit was immunized on day 1 with 150 µg of murine B7RP1 protein emulsified in an equal volume of Hunters Titer Max complete adjuvant. Further boosts  
10 (days 14 and 28) were performed by the same procedure. Antibody titers were monitored by EIA. After the second boost, the antisera revealed moderate antibody titers. A 30ml production bleed was then obtained from each animal. This was repeated each week for 6 weeks.  
15 Polyclonal antibodies were then purified by protein-A agarose chromatography, followed by negative selection Fc protein affinity chromatography and positive selection by B7RP1-Fc affinity chromatography.

20   Rat anti-murine B7RP1 monoclonal antibody preparation

          Rat anti-murine B7RP1 monoclonal antibodies were generated as described in Practical Immunology, second edition (1980; L. Hudson and F.C. Hay; Blackwell Scientific Publications; St. Louis, MO). Briefly, Lou  
25 rats (Harlan; Indianapolis, IN) were injected intraperitoneally with muB7RP1-Fc fusion protein emulsified in Freund's Adjuvant at 4 week intervals. Three days prior to fusion, rats were boosted intravenously with soluble muB7RP1. On the day of  
30 fusion, the animal was sacrificed under carbon dioxide and the spleen removed aseptically. Single cell suspension was generated using a tissue stomacher. Both splenocytes and Y3-Ag1.2.3 myeloma cells (American Type Culture Collection; Rockville, MD) were washed in  
35 serum-free media then fused by the addition of

polyethylene glycol (PEG 1500; Boehringer Mannheim Biochemicals; Indianapolis, IN). The cells were rinsed once, resuspended in serum-containing media, and plated into 96-well tissue culture plates. Ten to 12 days later, media from each well was tested for specific antibody to B7RP1 via a direct Enzyme-linked Immunosorbent Assay (EIA). Cells from wells indicating potential binding were grown to 10 ml cultures and frozen in liquid nitrogen. Media from each culture was further tested in flow cytometry and in a functional T-cell proliferation assay. Those determined to be of interest by these methods were plated to single cell colonies, selected again by EIA, and final cell lines maintained for antibody generation. Antibodies were purified from the cell media by protein A agarose chromatography.

#### T-cell preparation and T-cell proliferation assay

T-cells from the spleens of C57Bl/6 mice (8-12 week-old females, Charles River Laboratories) were purified by negative selection through a murine T-cell enrichment column (R&D Systems). The T-cells were then either used directly or further purified by antibody and complement lysis as follows. Cells were resuspended ( $2.5 \times 10^6$  cells/ml) in RPMI medium containing antibodies (all at 10  $\mu$ g/ml and from Pharmingen) against murine CD11b (Clone M1/70), NK-1.1 (Clone PK136), CD8a (Clone 53-6.7), I-A<sup>b</sup> (Clone M5/114.15.2), CD11c (Clone HL3), and the B220 antigen (Clone RA3-6B2). The cells were then incubated on ice for 30 min, pelleted at 1200 rpm, resuspended in 4:1 vol/vol of RPMI: rabbit complement (Sigma, #S-7764), and incubated for an additional 30 min at 37°C. The cells were pelleted again, and the complement treatment was repeated. Before plating, the cells were washed with RPMI containing 10% FCS. U-bottomed 96 well

plates were coated with an anti-CD3 antibody (Clone 145-2C11, Pharmingen) at concentrations ranging between 0 and 1.2 µg/ml, and anti-human IgG Fab<sub>2</sub> (Sigma, 12.5 µg/ml) overnight at 4°C, followed by a 6-9 hr incubation at 37°C. T-cells (1x 10<sup>5</sup>/well) were cultured in the absence or presence of various Fc fusion proteins for 48 hr and were pulsed during the last 18 hours with 1 µCi of <sup>3</sup>H-thymidine. Control Fc proteins included a fusion protein of OPG and Fc and a nonfused Fc protein fragment. The cells were then harvested and the incorporated radioactivity was counted. B7RP1-Fc co-stimulates T-cells to proliferate in a dose-dependent fashion (Fig. 15a), and an anti-B7RP1-Fc antibody specifically inhibits this co-stimulation dose-dependently (Fig. 15b).

#### Example 18

Inhibitors of the CRP1/B7RP1 pathway decrease the onset of rheumatoid arthritis induced by collagen.

Collagen-induced arthritis (CIA) is an animal model of autoimmune polyarthritis in rodents and primates that has many similarities with rheumatoid arthritis in humans. Immunization with heterologous species of type II collagen (CII) induces an autoimmune response to CII that leads to the development of CIA in susceptible mouse strains. Congenic strains of mice with H-2<sup>r</sup> and H-2<sup>q</sup> are highly susceptible to CIA. CIA is mediated by the synergistic effects of both CII-reactive T-cells and antibodies. Porcine CII (Nabozny et al., Autoimmunity 20, 51-58 (1995)) was dissolved in 0.01N acetic acid at a concentration of 2mg/ml and then was emulsified at a 1:1 ratio with CFA (Difco). Arthritis susceptible B10.RIII (H-2<sup>r</sup>) mice (Jackson

Laboratories, Bar Harbor, ME) were immunized with 100  $\mu$ l of emulsion intradermally at the base of the tail. Mice were monitored 2-3 times per week for the development of arthritis. Arthritis severity was determined using a grading system for each paw as follows: 0: no arthritis; 1: redness or swelling in 1-3 toes; 2: severe swelling of paw; 3: joint ankylosis. The score of each limb was summed to give a severity range from 0 to 12 for each animal.

Mice were injected with 100ug (in 200  $\mu$ L) of protein intraperitoneally twice per week. The treatment was begun 1 day after immunization with porcine CII and was stopped at day 52 post-immunization. The experiment was conducted in treatment groups of 10 mice, and animals with scores of 1 or above were scored as positive. The results are shown in Figure 16 and Table 1.

TABLE 1

Effect of CRP1, B7RP1, CTLA-4, and B7.2 Fc fusion proteins on the onset of arthritis

|    | <u>Treatment groups</u> | <u>Mean+/-s.d. day of onset</u> |
|----|-------------------------|---------------------------------|
| 25 | CTLA4-Fc                | 60.0 $\pm$ 0.0                  |
|    | CRP1-Fc                 | 48.9 $\pm$ 13.2                 |
| 30 | B7.2-Fc                 | 28.4 $\pm$ 14.1                 |
|    | B7RP1-Fc                | 33.9 $\pm$ 16.6                 |
|    | PBS                     | 37.7 $\pm$ 17.1                 |

In mice treated with CRP1-Fc fusion protein, the onset of arthritic symptoms were delayed by approximately 10 days as compared to the PBS treated mice. This demonstrates that the inhibition of the

CRP1/B7RP1 pathway can alleviate disease symptoms in this mouse model of rheumatoid arthritis.

Mice treated with B7RP1-Fc or B7.2-Fc showed an earlier onset of disease (Table 1 and Figure 16a) with an increase in arthritic severity (Figure 16b) as compared to the PBS-treated controls. This indicates that the B7RP1-Fc fusion protein enhances the T-cell immune response. Such activity may be useful in generating anti-tumor immunity *in vivo*.

The opposite effects by CRP1-Fc and B7RP1-Fc in this mouse model of rheumatoid arthritis indicate that the pathway can be manipulated to either enhance or inhibit the disease progression. Targeting the CRP1 protein with soluble B7RP1-Fc enhances the disease, while the interaction of soluble CRP1-Fc with B7RP1 inhibits the disease symptoms.

#### Example 19

B7RP1-Fc induces an inflammatory bowel disease phenotype in transgenic mice.

Persistent overexpression of the B7-related protein (B7RP1-Fc) in 22-to-25-week-old transgenic mice (Example 12) induces a striking phenotype of inflammatory bowel disease (IBD) with marked thickening and chronic inflammation of the small and large bowels (enterocolitis) and weight loss in some animals. Histologically, the most severe inflammatory changes were found in the proximal and distal colon, with milder changes in the small intestine. The proximal colon was markedly thickened with fissuring ulceration, transmural inflammation, and hypertrophy of the colonic mucosa, while the distal colon had diffuse mucosal hypertrophy (or focal erosion and glandular atrophy)

without ulceration. The proximal small intestine had mild to marked mucosal hypertrophy with milder inflammatory changes, while the distal small intestine (ileum) had mild mucosal hypertrophy in some animals and atrophy in other mice. The intestinal changes were most severe and consistently found in the female B7RP1 transgenic mice, but were also observed in several of the male transgenic mice in this study.

It is interesting to note that the histologic features found in the proximal colon, including the fissuring ulceration and the transmural chronic granulomatous inflammation with multinucleated giant cells, more closely resemble those seen in Crohn's disease than ulcerative colitis in humans. Morphologically, this colitis also mimics the IBD described in mice deficient in interleukin-10, which develop wasting, anemia, and enterocolitis affecting their entire intestinal tract (Kuhn et al. 1993; Sartor 1995; Leach et al. 1999). As in the IL-10 knockout mice, the initial changes in the B7RP1-Fc transgenic mice consist of mild, focal infiltrates of inflammatory cells in the lamina propria without colonic epithelial hyperplasia (Example 13). In older mice, the affected colonic segments become thickened due to glandular hypertrophy/hyperplasia and chronic inflammation. The proximal and distal colons of the B7RP1-Fc mice had moderate to severe colitis with histologic features of inflammatory bowel disease (IBD). The affected segments of the proximal colon (Figure 17B-17D) were diffusely thickened, due to prominent glandular epithelial hypertrophy and hyperplasia with elongation and dilatation of the mucosal glands (Fig. 17B), which had increased numbers of mitotic figures and rare crypt abscesses, but retained goblet cells with mucin (Fig. 17D). The mucosa had diffuse chronic inflammation in the lamina propria, which in some animals extended

transmurally to involve the underlying layers of the gut wall, including the submucosa, muscularis, serosa, and the adjacent mesenteric fat tissue (Fig. 17B-17C). The inflammatory infiltrates consisted of lymphocytes (predominantly CD3+, CD44+ T-cells), plasma cells, and epithelioid macrophages (Fig. 17F) mixed with some neutrophils and occasional multinucleated giant cells (Fig. 17E), characteristic of chronic granulomatous inflammation. Lymphoid aggregates (mostly B220+ cells mixed with small numbers of CD3+ cells) were also present in the mucosa and around smaller blood vessels in the submucosa and deeper layers, including mesenteric fat (Fig. 17C). The lumen contained mucopurulent or mucous exudate (Fig. 17D). Severe evidence of colitis, with multifocal fissuring ulceration of the mucosa and transmural inflammation (Fig. 17B-17C), was found in these B7RP1-Fc transgenic mice.

The distal colon of the B7RP1-Fc transgenic mice was also diffusely thickened and hyperplastic with elongation, basophilia, and dilatation of the colonic glands (Fig. 18B-18G), some of which contained crypt abscesses (Fig. 18D and 18F) and mucus. The lamina propria had a mild diffuse inflammatory infiltrate of lymphocytes (predominantly CD3+, CD44+ cells, particularly in the superficial mucosa; Fig. 18E), as well as plasma cells and focal aggregates of epithelioid macrophages mixed with some neutrophils. Lymphoid aggregates (of predominantly B220+ cells; Fig. 18D and 18F) were also scattered throughout the mucosa. The small intestine of B7RP1-Fc transgenic mice had more variable changes, including mild to focally marked mucosal and crypt hypertrophy and hyperplasia (Fig. 19B and 19D with crypt/villus ratios ranging from 1:4 to 1.5:1, as compared to 1:10 in the control mice) accompanied by a predominantly lymphoplasmacytic

The B7RP1-Fc fusion protein acts to activate cells that are responsible for eliciting a phenotype very similar to that of human Crohn's disease. This indicates that the cells that may be responsible for the inflammation in Crohn's disease are activated by the B7RP1-Fc fusion protein. Soluble protein, antibody or small molecule inhibitors of B7RP1 may therefore be useful in inhibiting IBD.

25                   The B7RP1-Fc fusion protein  
                  inhibits tumor growth in mice

Exponentially growing Meth A sarcoma cells were implanted by intradermal injection of 0.5 million cells in the abdomen of Balb/c mice on day 0. On day 7, when the tumors reached ~ 100 mm<sup>3</sup>, the mice were treated with either vehicle



(PBS) or B7RP1-Fc (8mg/kg), subcutaneously in the neck on days 7, 10, 14, and 17. The bidimensional diameters of the tumors were measured by calipers and the tumor volume (in mm<sup>3</sup>) was estimated using the formula: Tumor volume = [(width)<sup>2</sup> x length] / 2]. The tumor growth was monitored up to day 28. Each group had eight mice.

The Meth A sarcoma growth pattern of the control tumor was bi-phasic: a slow initial phase was followed by a relatively rapid exponential phase. In B7RP1-Fc treated mice, the growth of the tumor was significantly slower in the rapid exponential phase. On day 28, the average volumes of the control and B7RP1-Fc treated mice were 1410 mm<sup>3</sup> and 580 mm<sup>3</sup>, respectively (Figure 20). Therefore, B7RP1-Fc treatment inhibited tumor growth significantly in this model. The data strongly suggest the beneficial therapeutic utility of the soluble B7RP1-Fc protein, and other activators of the B7RP1/CRP1 pathway, in the treatment of immunogenic tumors.

Immunologic anti-tumor activity is closely associated with cytolytic T-lymphocyte (CTL) function. Consistently, the B7RP1-Fc protein is expressed on cytolytic CD8+ T-cells (Example 9, Figure 6). These data strongly support B7RP1 functions on cytolytic CD8+ T-cells. B7RP1-Fc, or other stimulators of the B7RP1/CRP1 pathway, may therefore be used to enhance cytolytic T-cell and cellular immune functions for a number of non-cancer-related indications.

#### Example 21

Inhibition of human B7RP1 activity in vitro

DEPT "0243260

To determine if human B7RP1 has positive co-stimulatory properties, we tested cells expressing human B7RP1 and human B7RP1-Fc fusion protein in T-cell proliferation assays. The human B7RP1-Fc fusion protein was constructed by fusing gene sequences corresponding to amino acids 1 to 247 to a partial human IgG1 gene sequence (Example 14). The human CRP1-Fc fusion protein was constructed by fusing gene sequences corresponding to amino acids 1 to 146 to a partial human IgG1 gene sequence (Example 2). The methods of construction, expression and purification of both fusion proteins were conducted as described in Example 7. B7RP1-Fc demonstrated co-stimulatory activities that are dependent on anti-CD3 stimulation (Figure 21a). In addition, this activity can be specifically inhibited with soluble CRP1-Fc protein (Figure 21b). Similar co-stimulatory effects were obtained using CHO cells that express membrane-bound, human B7RP1, containing the entire coding sequence (Figure 21c).

The production of cytokines by human T-cells under the above *in vitro* proliferation conditions was determined. Supernatants from T-cell cultures stimulated for 48 and 72 hours were analyzed for IL-2, IL-10, and IFN-gamma by ELISA according to the manufacturer's specifications (BioSource International). The IFN-gamma and IL-10 levels were significantly increased; however, unlike the case with CD28 co-stimulation, IL-2 was not notably induced (Figure 21d). The increased levels of IFN-gamma, a Th1 cytokine, correlate with the B7RP1 functions to increase IgG2a, as described in Example 13.

*In vitro* T-cell co-stimulation assays were conducted as follows. Highly purified human T-cells (>98% CD3+) were isolated by negative

selection of fresh or thawed, adherence depleted PBMC using mAb labeled magnetic beads (Miltenyi Biotec). T-cells ( $1 \times 10^5$  cells/well) were cultured in triplicate wells in 96 well plates in 200  $\mu$ l/well RPMI + 10% FCS. To evaluate B7RP1-Fc co-stimulation, various concentrations of anti-CD3 (Pharmingen) and 10  $\mu$ g/ml anti-human IgG Fc (Sigma) in 100  $\mu$ l 1X PBS were pre-coated onto U bottom plates by an incubation at 4°C overnight. The unbound anti-CD3 and anti-human IgG Fc were removed, and the cells were cultured in the presence or absence of various concentrations of B7RP1-Fc, OPG-Fc control or anti-CD28 (Pharmingen). For CRP1-Fc inhibition of B7RP1-Fc co-stimulation, T-cells were cultured in 0.33  $\mu$ g/ml anti-CD3 and 10  $\mu$ g/ml anti-human IgG Fc pre-coated wells with 0.5  $\mu$ g/ml B7RP1-Fc in the presence of serially diluted CRP1-Fc or OPG-Fc, starting at 10  $\mu$ g/ml. To evaluate co-stimulation by CHO cells expressing B7RP1, T-cells were cultured in flat bottom plates with various concentrations of soluble anti-CD3 in the presence or absence of various amounts of mitomycin-C treated CHO B7RP1 cells or CHO vector cells. To test for T-cell proliferation, cultures were pulsed with 1 uCi/well [ $^3$ H]TdR during the last 18 hrs of a 72 hr culture. T-cell proliferation was determined by [ $^3$ H]TdR incorporation. The results of one representative experiment from three random donors are expressed as mean CPM incorporated  $\pm$  SD. For analyses of cytokine production, cells were cultured for 48 and 72 hours and supernatants were collected for ELISA.

These experiments show that the extracellular portion of human B7RP1, as described

in Example 14, when fused to a human Fc fragment, can co-stimulate T-cells in vitro. This co-stimulation is inhibited by CRP1-Fc and thus demonstrates how a soluble inhibitor of human B7RP1 may function. In vitro assays, such as that described here using human B7RP1 and CRP1, could be used to screen for antibody, soluble protein, peptibody, or small molecule inhibitors of B7RP1/CRP1 activity.

#### Example 22

B7RP1 transgenic mice have increased IgE levels

Transgenic mice described in Example 12 were analyzed for serum IgE antibodies. Orbital sera from B7RP1-Fc transgenic mice and littermate controls were analyzed for IgE by an ELISA protocol. 96-well polystyrene EIA plates (Coster, Cambridge, MA) were coated with 50  $\mu$ l of a 5  $\mu$ g/ml solution of rat anti-mouse IgE antibody (BD Pharmingen, San Diego, CA) in 50 mM Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> buffer (pH 9.6 at room temperature for 2 hr then at 4°C overnight. The subsequent procedures were conducted at room temperature. After blocking with assay diluent (1% BSA, 1% normal goat serum, and 0.2% tween-20 in PBS) for 30 min, serum samples and mouse IgE standards (BD Pharmingen) were added into the wells, and the plates were incubated for 2 hr. The wells were washed 3 times with KPL wash buffer (KPL, Gaithersburg, MD). Biotin-conjugated goat anti-rat antibody (BD Pharmingen) was added and incubated for 1 hr. After washing 3 times with KPL wash buffer, horseradish peroxidase-conjugated streptavidin (BD Pharmingen) was added to the wells, and the plates were incubated for 30 min. After the wells were washed 5 times with KPL wash buffer, TMB-hydrogen peroxide substrate solution (KPL) was added, and the plates were

5 approximately three times higher than those in the littermate controls (Figure 22). This increase in the IgE serum levels indicates that B7RP1 regulates IgE expression.

### IgE-mediated response of CRP1 knockout mice

The CRP1 gene in mice was disrupted by deleting a genomic fragment corresponding to nucleotides 318-591 of the murine CRP1 cDNA sequence (see SEQ ID NO:\_\_\_). The murine CRP1 gene was isolated from a 129J library using the full-length (800 bp) cDNA probe (Yoshinaga et al. Nature 402, 827-832 (1999)). The targeting vector, which replaced a 2.8kb genomic fragment with a neomycin resistance (neo) cassette in sense orientation relative to CRP1 transcription, was electroporated into E14 embryonic stem (ES) cells (129/Ola, available from the American Type Culture Collection, Manassas, Va under accession no. CRL-1821). After G418 selection, homologous recombinants were identified by PCR using the primer pair GAG ACT CAT GCT GTG GTT TCA GG (SEQ ID NO:\_\_\_) and TTC GCC AAT GAC AAG ACG CTG G (SEQ ID NO:\_\_\_) and verified by Southern blotting. Chimeric mice generated from CRP1<sup>+/-</sup> ES clones were crossed with C57BL6 females to produce CRP1<sup>+/-</sup> mice. Germline transmission of the CRP1 mutation was assessed by PCR and Southern blot analysis of tail DNA. CRP1<sup>-/-</sup> mice generated by the intercrossing of heterozygous offspring were born at the expected Mendelian frequency and were viable, fertile and of normal size. To verify that the CRP1

5 CD4<sup>+</sup> and CD8<sup>+</sup> wild type T-cells, but was undetectable on  
CRP1<sup>-/-</sup> T-cells.

10 immunized with 200 µg of nitrophenol conjugated  
ovalbumin (NP-OVA; Biosearch Technologies, Inc.)  
adsorbed to alum intraperitoneally. Blood was  
collected from mouse tails every week following  
immunization and the levels of nitrophenol (NP)-  
15 specific IgM and IgG<sub>1</sub> as well as the levels of OVA-  
specific IgE were assessed in the serum.

coated with NP(23)-bovine serum albumin at 50 µg/ml in PBS or carbonate buffer pH 9.2 (Sigma). The arbitrary values obtained by using the SOFTmaxPRO (Molecular Devices) ELISA analysis program were based on the titration curve of the isotype standard used on each plate. Levels of ovalbumin-specific IgE were detected using an antigen-capture (biotinylated ovalbumin) ELISA method as previously described (Stampfli et al. Am J. Respir. Cell Mol. Biol. 21, 317-326 (1999)). The ELISA was standardized using serum obtained from mice sensitized to ovalbumin according to a conventional intraperitoneal sensitization model (Ohkawara et al. Am. J. Respir. Cell Mol. Biol. 16, 510 (1997)).

The levels of NP-specific IgM were normal (day 7); however, a significant reduction in the levels of NP-specific IgG, at day 14 and 21 post-immunization,

Figure 1 consists of four panels (a, b, c, d) showing precipitation and runoff anomalies in the Amazon basin. The maps are oriented with North at the top. A color scale at the bottom indicates precipitation anomalies in mm, ranging from -100 (dark blue) to 100 (dark red), with 0 in white. Panel (a) shows precipitation anomalies for 1997-1998, 1998-1999, and 1999-2000. Panel (b) shows runoff anomalies for the same periods. Panel (c) shows the difference in precipitation anomalies between 1997-1998 and 1998-1999. Panel (d) shows the difference in runoff anomalies between 1997-1998 and 1998-1999. The maps show significant positive anomalies (red) in the central and eastern Amazon during 1997-1998, which are reduced or reversed in subsequent years.

indicating that T-cell-dependent antibody production was severely impaired in CRP1<sup>-/-</sup> mice. Furthermore, we observed that OVA-specific IgE were completely undetectable in the serum of ICOS<sup>-/-</sup> mice at day 21 and 28 post-immunization, whereas isotype switching occurred in both heterozygous and wild type control mice, as demonstrated by the presence of IgE in the serum of these animals. (see Table 3). These findings indicate that ICOS plays a role of primary importance in controlling T-B cell collaboration and switching of IgG<sub>1</sub> and IgE isotype classes.

Table 3  
IgE isotype switching in CRP1<sup>-/-</sup> mice

| Days post-immunization | CRP1 <sup>+/+</sup> | CRP1 <sup>+/-</sup> | CRP1 <sup>-/-</sup> |
|------------------------|---------------------|---------------------|---------------------|
| Day 21                 | 4/8                 | 5/9                 | 0/7                 |
| Day 28                 | 4/6                 | 8/9                 | 0/8                 |

Example 24  
Effects of B7RP1 and B7.2  
in a Contact Hypersensitivity Model

In order to induce contact hypersensitivity, female Balb/c mice (9 to 14 weeks old; Charles River Laboratories, Wilmington, MA) were first sensitized by applying a 1% solution of oxazolone (Sigma, St. Louis, MO) in acetone and olive oil onto the shaved skin of the abdomen. Seven days after sensitization, mice were challenged (day 0) by applying the oxazolone solution onto the right ear. The acetone and olive oil solvent was applied at the same time onto the left ear as a control. The thickness of the ears was measured daily

with a micrometer (Mitutoyo, Kawasaki, Japan) starting immediately before challenge. The difference in ear thickness ( $\Delta$ ET) between right and left ear was used to express the results (McHale et al. J. Immunol 162, 1648 (1999)).

It has been previously observed that administration of B7RP1-Fc fusion protein in this model exacerbates contact hypersensitivity both at the time of sensitization (induction of the primary immune response) or at the time of challenge (induction of the secondary immune response), although the effects are more pronounced when administration occurs at the time of challenge (Yoshinaga et al. Nature 402, 827-832 (1999)).

In order to study the combined effects of B7RP1-Fc and B7.2-Fc on contact hypersensitivity, B7RP1-Fc and B7.2-Fc were given at two different doses either alone or in combination around the time of challenge. At high dose (2 mg/Kg of B7RP1-Fc alone, 2 mg/Kg of B7.2-Fc alone, and 1 mg/Kg of each in combination), B7RP1-Fc, B7.2-Fc, and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET compared to Fc (2mg/Kg) from day 3 (Fig. 23A). At this dose B7RP1-Fc and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET more than B7.2-Fc from day 4 (Fig. 23A). B7RP1-Fc+B7.2-Fc also increased  $\Delta$ ET more than B7RP1-Fc from day 4 (Fig. 23A). At low dose (0.4 mg/Kg of B7RP1-Fc alone, 0.4 mg/Kg of B7.2-Fc alone, and 0.2 mg/Kg of each in combination), B7RP1-Fc and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET compared to Fc (0.4 mg/Kg) from day 4, while B7.2-Fc did not significantly change  $\Delta$ ET (Fig. 23B). Also at this dose B7RP1-Fc and B7RP1-Fc+B7.2-Fc increased  $\Delta$ ET more than B7.2-Fc, and B7RP1-Fc+B7.2-Fc more than B7RP1-Fc (Fig. 23B). Thus, half of full doses of B7RP1-Fc combined with half of full



doses of B7.2-Fc increased  $\Delta$ ET on days 4 to 7 more than full doses of B7RP1-Fc alone or of B7.2 alone, indicating that B7RP1-Fc and B7.2-Fc synergistically interacted.

5

\* \* \*

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed.

10

15

00347 "0443460

## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
- a) the nucleotide sequence as set forth in Figure 1A (SEQ ID NO: 1);
  - b) the nucleotide sequence encoding the polypeptide from residues 1-200 or from residues 21-200 as set forth in Figure 1A (SEQ ID NO: 1);
  - c) a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in Figure 1A (SEQ ID NO: 1);
  - d) a naturally occurring allelic variant or alternate splice variant of any of (a), (b) or (c );
  - e) a nucleotide sequence complementary to any of (a), (b) or (c );
  - f) a nucleotide sequence of (b), (c ) or (d) encoding a polypeptide fragment of at least about 25, 50, 75, 100, or greater than 100 amino acid residues;
  - g) a nucleotide sequence of (a), (b) or (c ) comprising a fragment of at least about 10, 15, 20, 25, 50, 75, 100, or greater than 100 nucleotides; and
  - h) a nucleotide sequence which hybridizes under stringent conditions to any of (a)-(g).

2. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- a) the nucleotide sequence as set forth in Figure 2A (SEQ ID NO: 11) or Figure 3A (SEQ ID NO: 6) or Figure 12A (SEQ ID NO: 16);
- b) the nucleotide sequence encoding the polypeptide as set forth in Figure 2A (SEQ ID NO: 6) from residues 1-322 or from residues 47-322, or as set

forth in Figure 3A (SEQ ID NO: 11) from residues 1-288 or from residues 19-288, 20-288, 21-288, 22-288, 24-288, or 28-288 or as set forth in Figure 12A from residues 1-302, or from residues 19-302, 20-302, 21-302, 22-302, 24-302 or 28-302;

c) a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in Figure 2A (SEQ ID NO: 6) or Figure 3A (SEQ ID NO: 11) or Figure 12A (SEQ ID NO: 6);

d) a naturally occurring allelic variant or alternate splice variant of any of (a), (b) or (c );

e) a nucleotide sequence complementary to any of (a), (b) or (c );

f) a nucleotide sequence of (b), (c ) or (d) encoding a polypeptide fragment of at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

g) a nucleotide sequence of (a), (b) or (c ) comprising a fragment of at least about 10, 15, 20, 25, 50, 75, 100, or greater than 100 nucleotides; and

h) a nucleotide sequence which hybridizes under stringent conditions to any of (a)-(g).

3. The nucleic acid molecule of Claims 1 or 2 wherein the nucleotide sequence is operably linked to an expression control sequence.

4. A host cell comprising the nucleic acid molecule of Claim 2.

5. The host cell of Claim 3 which is a eucaryotic cell.

6. The host cell of Claim 3 which is a procaryotic cell.

7. A process for producing a polypeptide comprising growing a culture of the host cell of Claim 3 in suitable culture medium and isolating the polypeptide from the culture.

5

8. A polypeptide produced by the process of Claim 7.

9. A polypeptide encoded by the nucleic acid molecule of Claim 1.

10

10. A polypeptide encoded by the nucleic acid molecule of Claim 2.

15

11. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

a) the amino acid sequence as set forth in Figure 1A (SEQ ID NO: 2);

b) the mature amino acid sequence as set forth in Figure 1A (SEQ ID NO: 2) comprising a mature amino terminus at residue 21;

c) a fragment of the amino acid sequence set forth in Figure 1A (SEQ ID NO: 2) comprising at least about 25, 50, 75, 100, or greater than 100 amino acid residues;

25

d) an ortholog of (a), (b) or (c); and

e) an allelic variant or alternative splice variant of (a), (b), (c) or (d).

30

12. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

a) the amino acid sequence as set forth in Figure 2A (SEQ ID NO: 7) or Figure 3A (SEQ ID NO: 12) or Figure 12A (SEQ ID NO: 17);

35

b) the mature amino acid sequence as set forth in Figure 2A (SEQ ID NO: 7) comprising a mature

5

10

e) an allelic variant or alternative splice  
f) (a), (b), (c) or (d).

15

20

15. The antibody of Claim 13 which is a human

25

17. The antibody or fragment of Claim 13 which binds B7RP1 or to a B7RP1 extracellular domain.

30

35

20. A polypeptide comprising a derivative of the polypeptide of Claims 9, 10, 11 or 12.

21. The polypeptide of Claim 20 which is covalently modified with a water-soluble polymer.

22. A fusion polypeptide comprising the polypeptide of Claims 9, 10, 11 or 12 fused to a  
10 heterologous amino acid sequence.

23. The fusion polypeptide of Claim 22 wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

24. A method for treating, preventing or ameliorating a T-cell mediated disorder comprising administering to an animal the polypeptide of Claims 9, 10, 11 or 12.

25. A method of diagnosing a T-cell mediated disorder or a susceptibility to a T-cell mediated disorder in an animal comprising:

a) determining the presence or amount of  
25 expression of the polypeptide of Claims 9, 10, 11 or  
12; and

b) diagnosing a T-cell mediated disorder or a susceptibility to a T-cell mediated disorder based on the presence or amount of expression of the polypeptide.

26. A method of identifying a test molecule which binds to a polypeptide comprising:

a) contacting the polypeptide of Claims 9,  
35 10, 11 or 12 with a test molecule; and

b) determining the extent of binding of the polypeptide to the test molecule.

27. The method of Claim 26 further comprising  
5 determining the activity of the polypeptide when bound to the compound.

28. A method of regulating T-cell activation or proliferation in an animal comprising administering to  
10 the animal the nucleic acid molecule of Claims 1, 2 or 3.

29. A transgenic non-human mammal comprising the nucleic acid molecule of Claim 3.  
15

30. A method of suppressing an immune response in an animal comprising administering to the animal an antagonist of CRP1 or B7RP1.

31. The method of Claim 30 wherein the antagonist is an antibody which binds B7RP1.  
20

32. A method of decreasing IgE production in an animal comprising administering a B7RP1 antagonist or a CRP1 antagonist, or a combination thereof, in an amount  
25 effective to decrease IgE production.

33. A method of preventing or treating an IgE-mediated disorder comprising administering a  
30 therapeutically effective amount of a B7RP1 antagonist, or a CRP1 antagonist, or a combination thereof.

34. The method of Claims 32 or 33 wherein the B7RP1 antagonist is an antibody which binds B7RP1 and  
35 partially or completely inhibits IgE production.

092840-12809

35. The method of Claims 32 or 33 wherein the CRP1 antagonist is an antibody which binds CRP1 and partially or completely inhibits IgE production.

5           36. The method of Claim 33 wherein the IgE-  
mediated disorder is asthma or an allergic disorder.

37. The method of Claims 32 or 33 further comprising administering an IgE antagonist.

10

38. The method of Claim 37 wherein the IgE antagonist is an anti-IgE antibody.

39. A method of enhancing an immune response  
15 comprising administering B7RP1, a B7RP1 agonist or a  
CRP1 agonist.

40. The method of Claim 39 further comprising administering a CD28 agonist.

20

41. The method of claim 39 further comprising administering B7.1 or B7.2 or both.

42. A method of treating cancer or viral infection  
25 comprising administering B7RP1 or an agonist of CRP1,  
optionally in combination with B7.1 or B7.2.



Figure 1 consists of 12 sub-graphs, labeled (a) through (l), arranged vertically. Each graph plots a different physiological parameter against time (0 to 10 minutes). The y-axis for all graphs is 'Arbitrary Units' ranging from 0 to 10. The x-axis is 'Time (min)' ranging from 0 to 10. Each graph shows a baseline period followed by a sharp increase at approximately 5 minutes, then a gradual decline.

- (a) Heart Rate (b/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (b) Blood Pressure (mmHg): Baseline ~80, peak ~95 at 5 min, then declines to ~85.
- (c) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (d) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (e) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (f) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (g) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (h) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (i) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (j) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (k) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.
- (l) Blood Flow (ml/min): Baseline ~5.5, peak ~8.5 at 5 min, then declines to ~6.5.

**FIGURE 1A**

45  
90  
135  
180  
225  
270  
315  
360  
405  
450  
495  
540  
585  
600





[illegible][illegible]



Figure 1 consists of 12 bar charts, each representing a different demographic or lifestyle category. Each chart compares the percentage of respondents across six age groups: 18-24, 25-34, 35-44, 45-54, 55-64, and 65+. The categories are as follows:

- 1. Gender:** Male (dark blue), Female (light blue).
- 2. Education:** High School (dark blue), Graduate (light blue).
- 3. Income:** Low (dark blue), Medium (light blue), High (medium blue).
- 4. Marital Status:** Single (dark blue), Married (light blue), Divorced (medium blue), Widowed (dark grey).
- 5. Employment:** Full-time (dark blue), Part-time (light blue), Unemployed (medium blue).
- 6. Health:** Good (dark blue), Fair (light blue), Poor (medium blue).
- 7. Living Arrangement:** Alone (dark blue), With Family (light blue), With Friends (medium blue).
- 8. Transportation:** Car (dark blue), Bus (light blue), Bike (medium blue), Walk (dark grey).
- 9. Communication:** Phone (dark blue), Internet (light blue), Email (medium blue).
- 10. Recreation:** TV (dark blue), Movies (light blue), Reading (medium blue), Exercise (dark grey).
- 11. Social:** Friends (dark blue), Family (light blue), Community (medium blue).
- 12. Volunteering:** Yes (dark blue), No (light blue).

The charts show varying trends across age groups. For example, in the 'Volunteering' chart, the 'Yes' response is highest in the 65+ age group, while in the 'Living Arrangement' chart, 'Alone' is most prevalent in the 18-24 group.

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | CGG | CTG | GGC | CTC | GGA | CTG | CTC | TTC | CTG | CTC | TTC | AGC | AGC |     | 45  |
| M   | R   | L   | G   | S   | P   | G   | L   | L   | F   | L   | L   | F   | S   | S   |     |
|     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |
| CTT | CGA | GCT | GAT | ACT | CAG | GAG | AAG | GAA | GTC | AGA | GCG | ATG | GTA | GGC | 90  |
| L   | R   | A   | *D  | *T  | *Q  | *E  | K   | *E  | V   | R   | A   | *M  | V   | G   |     |
|     |     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |
| AGC | GAC | GTG | GAG | CTC | AGC | TGC | GCT | TGC | CCT | GAA | GGA | AGC | CGT | TTT | 135 |
| S   | D   | V   | E   | L   | S   | C   | A   | C   | P   | E   | G   | S   | R   | F   |     |
|     |     |     | 35  |     |     |     |     |     | 40  |     |     |     |     | 45  |     |
| GAT | TTA | AAT | GAT | GTT | TAC | GTA | TAT | TGG | CAA | ACC | AGT | GAG | TCG | AAA | 180 |
| D   | L   | N   | D   | V   | Y   | V   | Y   | W   | Q   | T   | S   | E   | S   | K   |     |
|     |     |     | 50  |     |     |     |     |     | 55  |     |     |     |     | 60  |     |
| ACC | GTG | GTG | ACC | TAC | CAC | ATC | CCA | CAG | AAC | AGC | TCC | TTG | GAA | AAC | 225 |
| T   | V   | V   | T   | Y   | H   | I   | P   | Q   | N   | S   | S   | L   | E   | N   |     |
|     |     |     | 65  |     |     |     |     |     | 70  |     |     |     |     | 75  |     |
| GTG | GAC | AGC | CGC | TAC | CGG | AAC | CGA | GCC | CTG | ATG | TCA | CCG | GCC | GGC | 270 |
| V   | D   | S   | R   | Y   | R   | N   | R   | A   | L   | M   | S   | P   | A   | G   |     |
|     |     |     | 80  |     |     |     |     |     | 85  |     |     |     |     | 90  |     |
| ATG | CTG | CGG | GGC | GAC | TTC | TCC | CTG | CGC | TTG | TTC | AAC | GTC | ACC | CCC | 315 |
| M   | L   | R   | G   | D   | F   | S   | L   | R   | L   | F   | N   | V   | T   | P   |     |
|     |     |     | 95  |     |     |     |     |     | 100 |     |     |     |     | 105 |     |
| CAG | GAC | GAG | CAG | AAG | TTT | CAC | TGC | CTG | GTG | TTG | AGC | CAA | TCC | CTG | 360 |
| Q   | D   | E   | Q   | K   | F   | H   | C   | L   | V   | L   | S   | Q   | S   | L   |     |
|     |     |     | 110 |     |     |     |     |     | 115 |     |     |     |     | 120 |     |
| GGA | TTC | CAG | GAG | GTT | TTG | AGC | GTT | GAG | GTT | ACA | CTG | CAT | GTG | GCA | 405 |
| G   | F   | Q   | E   | V   | L   | S   | V   | E   | V   | T   | L   | H   | V   | A   |     |
|     |     |     | 125 |     |     |     |     |     | 130 |     |     |     |     | 135 |     |
| GCA | AAC | TTC | AGC | GTG | CCC | GTC | GTC | AGC | GCC | CCC | CAC | AGC | CCC | TCC | 450 |
| A   | N   | F   | S   | V   | P   | V   | V   | S   | A   | P   | H   | S   | P   | S   |     |
|     |     |     | 140 |     |     |     |     |     | 145 |     |     |     |     | 150 |     |
| CAG | GAT | GAG | CTC | ACC | TTC | ACG | TGT | ACA | TCC | ATA | AAC | GGC | TAC | CCC | 495 |
| Q   | D   | E   | L   | T   | F   | T   | C   | T   | S   | I   | N   | G   | Y   | P   |     |
|     |     |     | 155 |     |     |     |     |     | 160 |     |     |     |     | 165 |     |
| AGG | CCC | AAC | GTG | TAC | TGG | ATC | AAT | AAG | ACG | GAC | AAC | AGC | CTG | CTG | 540 |
| R   | P   | N   | V   | Y   | W   | I   | N   | K   | T   | D   | N   | S   | L   | L   |     |
|     |     |     | 170 |     |     |     |     |     | 175 |     |     |     |     | 180 |     |
| GAC | CAG | GCT | CTG | CAG | AAT | GAC | ACC | GTC | TTC | TTG | AAC | ATG | CGG | GGC | 585 |
| D   | Q   | A   | L   | Q   | N   | D   | T   | V   | F   | L   | N   | M   | R   | G   |     |
|     |     |     | 185 |     |     |     |     |     | 190 |     |     |     |     | 195 |     |
| TTG | TAT | GAC | GTG | GTC | AGC | GTG | CTG | AGG | ATC | GCA | CGG | ACC | CCC | AGC | 630 |
| L   | Y   | D   | V   | V   | S   | V   | L   | R   | I   | A   | R   | T   | P   | S   |     |
|     |     |     | 200 |     |     |     |     |     | 205 |     |     |     |     | 210 |     |
| GTG | AAC | ATT | GGC | TGC | TGC | ATA | GAG | AAC | GTG | CTT | CTG | CAG | CAG | AAC | 675 |
| V   | N   | I   | G   | C   | C   | I   | E   | N   | V   | L   | L   | Q   | Q   | N   |     |
|     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |     | 225 |     |
| CTG | ACT | GTC | GGC | AGC | CAG | ACA | GGA | AAT | GAC | ATC | GGA | GAG | AGA | GAC | 720 |
| L   | T   | V   | G   | S   | Q   | T   | G   | N   | D   | I   | G   | E   | R   | D   |     |
|     |     |     | 230 |     |     |     |     |     | 235 |     |     |     |     | 240 |     |

[illegible]

765  
810  
855  
864



# FIGURE 3B

|           |            |            |            |             |              |     |
|-----------|------------|------------|------------|-------------|--------------|-----|
| hB7RP1    | EKEVRAMVGS | DVELSCACPE | GSRFDLNDVY | VYWQTSESKT  | VVTYHIPQNS   | 50  |
| mB7RP1    | ETEVGAMVGS | NVVLSCIDPH | RRHFNLSGLY | VYWQIENPEV  | SVTTYLPYKS   | 50  |
| Consensus | E.EV.AMVGS | .V.LSC..P. | ...F.L...Y | VYWQ.....   | .VTY...P...S |     |
| hB7RP1    | SLENVDSRYR | NRALMSPAGM | LRGDFSLRLF | NVTPQDEQKF  | HCLVLSQ-SL   | 99  |
| mB7RP1    | PGINVDSYK  | NRGHLSDSM  | KQGNFSLYLK | NVTPQDTQEF  | TCRVFMTAT    | 100 |
| Consensus | ...NVDS.Y. | NR...S...M | ..G.FSL.L. | NVTPQD.Q.F  | .C.V.....    |     |
| hB7RP1    | GFQEVLSVEV | TLHVAANFSV | PVVSAPHSPS | Q-DELTFTCT  | SINGYPRPNV   | 143 |
| mB7RP1    | ELVKILEEVV | RLRVAANFST | PVISTSDSSN | PGQERTYTCM  | SKNGYPEPNL   | 150 |
| Consensus | .....L...V | .L.VAANFS. | PV.S...S.. | ...E.T.TC.  | S.NGY.P.N.   |     |
| hB7RP1    | YWINKTDNSL | LDQALQNDTV | FLNMRGLYDV | VSVLRIARTP  | SVNIGCCIEI   | 198 |
| mB7RP1    | YWINTTDNSL | IDTALQNNTV | YLNKLGLYDV | ISTLRLPWTS  | RGDVLCCEV    | 200 |
| Consensus | YWIN.TDNSL | .D.ALQN.TV | .LN..GLYDV | .S.LR...T.  | .....CC.EN   |     |
| hB7RP1    | VLLQQNLTVG | SQTGNDIGER | DKITENPVST | GEKNAATWSI  | LAVLCLLVVV   | 248 |
| mB7RP1    | VALHQNITSI | SQAESFTGNN | TKNPQETHNN | ELKVLV--PV  | LAVLAAAFV    | 248 |
| Consensus | V.L.QN.T.. | SQ...G..   | .K.....C.. | ...K...7... | LAVL.....V   |     |
| hB7RP1    | AVAIGWVCRD | RCLQHSYAG  |            |             |              | 267 |
| mB7RP1    | SFIIYR--RT | R-PHRSYTGP | KTVQLELTDH | A           |              | 276 |
| Consensus | ...I...R.  | R...SY.G.  | .....      |             |              |     |

003217" 02482260

Figure 4A

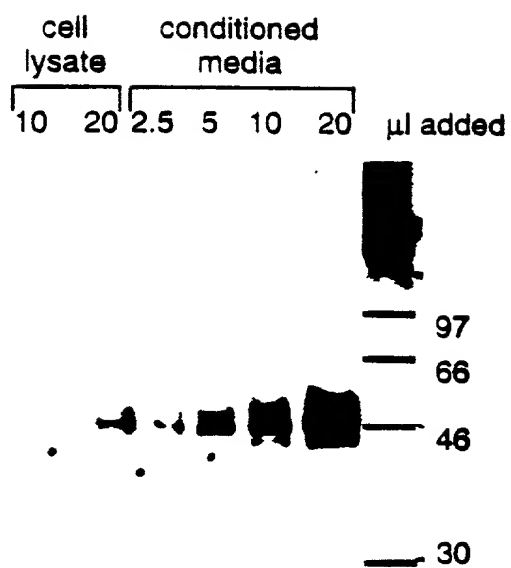


Figure 4B

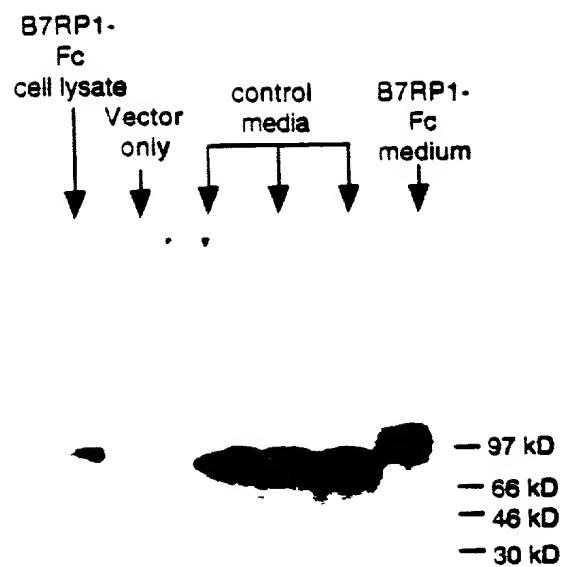




Figure 6

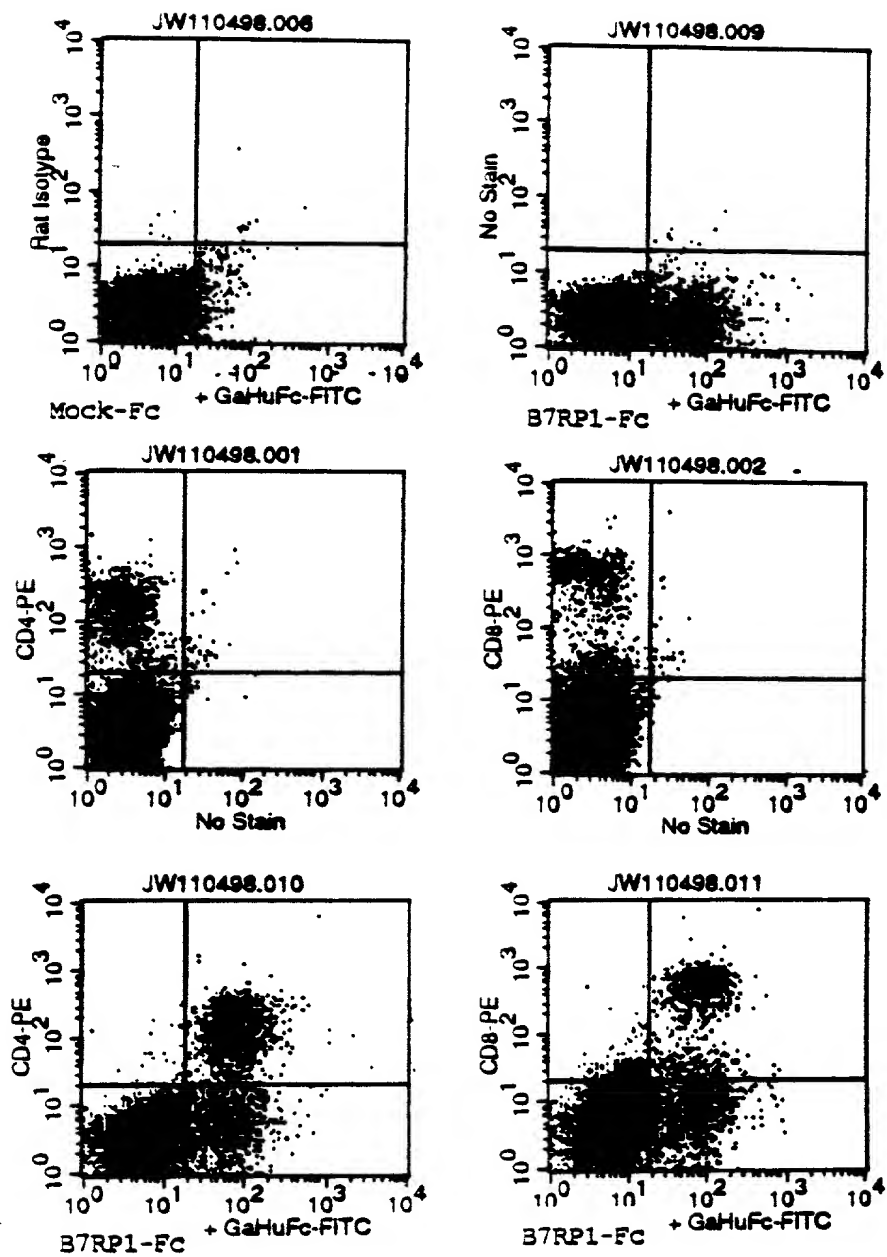


Figure 7

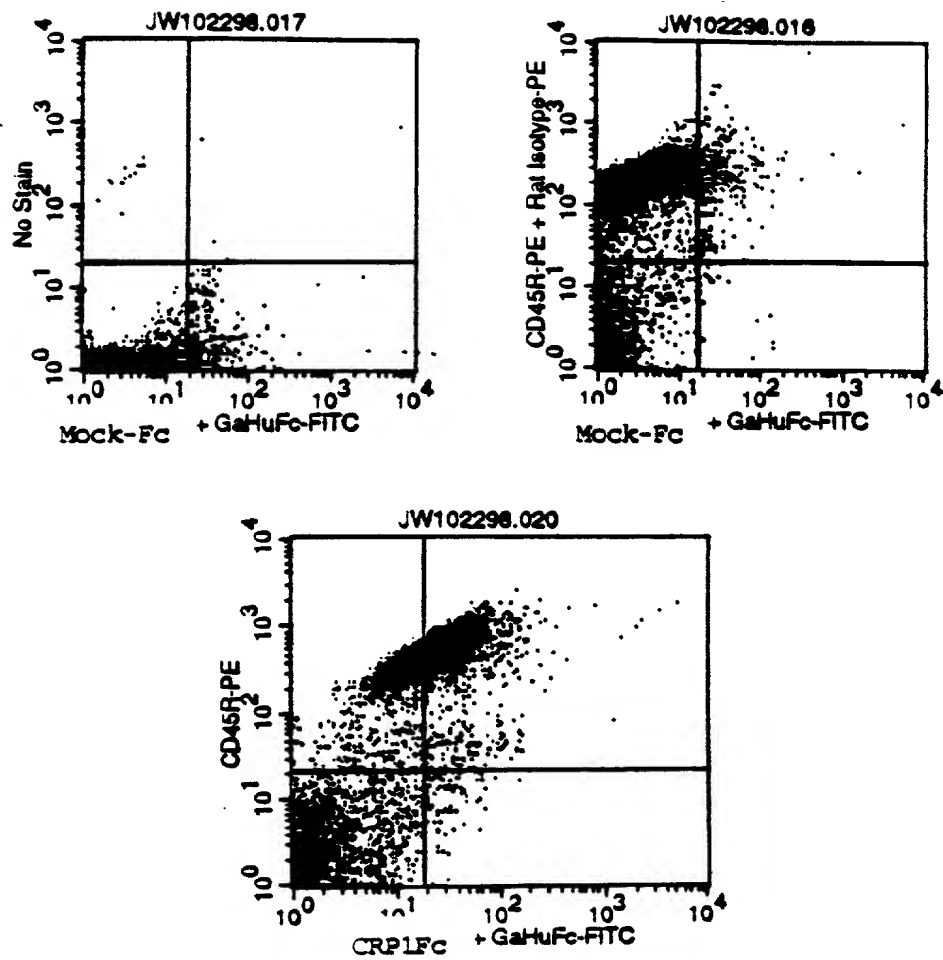


Figure 3

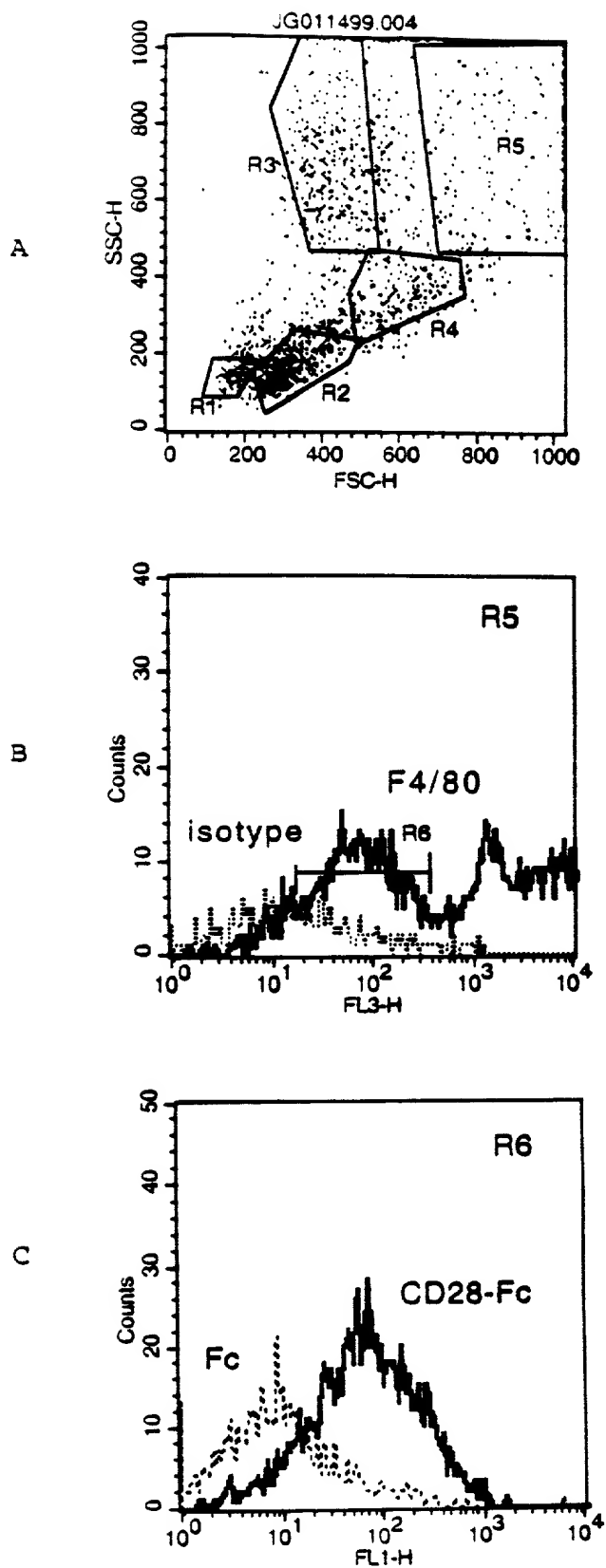


FIGURE 9

Con A stimulation of T-cells regulated  
by B7RP1-Fc fusion protein

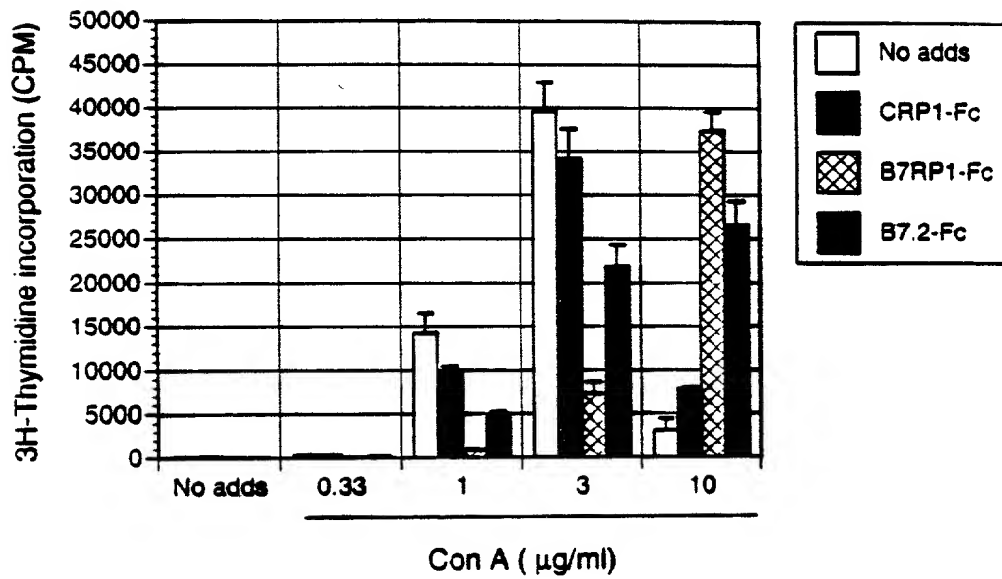




Figure 10

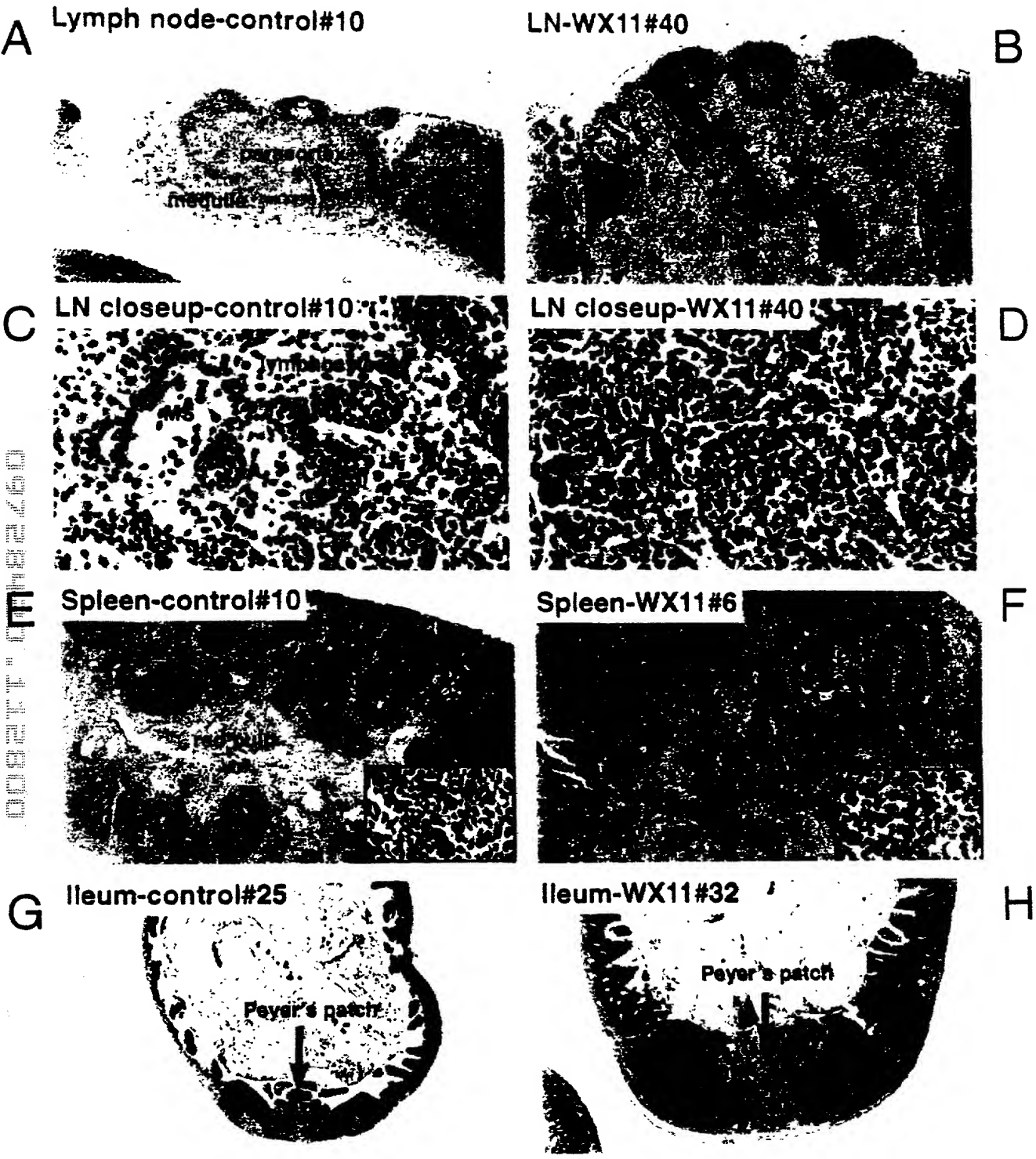


Figure 11

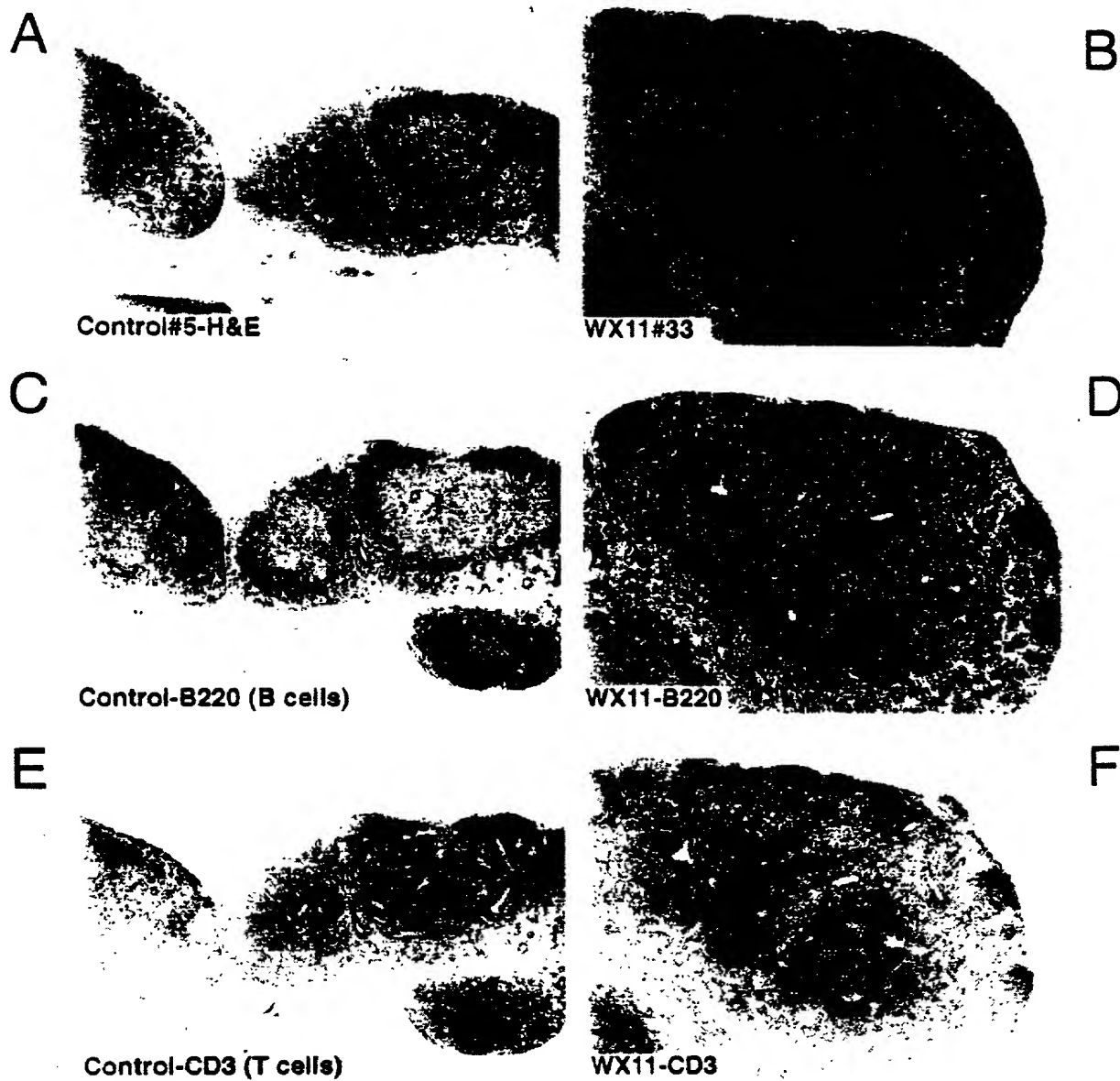


Figure 12A.

|  |      |
|--|------|
| GCTGGTACGCCTGCAGGTACCGGTCCGGAATTCCCGGGTTCGACCCACGCGTCCGCCCCACGCG | -138 |
| TCCGCGGGAGCGCAGTTAGAGCCGATCTCCCGCGCCCCGAGGTTGCTCCTCTCCGAGGTCTC   | -76  |
| CCGCGGCCCAAGTTCTCCGCGCCCCGAGGTCTCCCGCCCCGAGGTCTCCGCGGCCCGAGGT    | -14  |
| CTCCGCCCCGACC  | -1   |
| ATG CGG CTG GGC AGT CCT GGA CTG CTC TTC CTG CTC TTC AGC AGC      | 45   |
| M R L G S P G L L F L L F S S                                    |      |
| 5 10 15  |      |
| CTT CGA GCT GAT ACT CAG GAG AAG GAA GTC AGA GCG ATG GTA GGC      | 90   |
| L R A *D *T *Q *E K *E V R A *M V G                              |      |
| 20 25 30   |      |
| AGC GAC GTG GAG CTC AGC TGC GCT TGC CCT GAA GGA AGC CGT TTT      | 135  |
| S D V E L S C A C P E G S R F                                    |      |
| 35 40 45   |      |
| GAT TTA AAT GAT GTT TAC GTA TAT TGG CAA ACC AGT GAG TCG AAA      | 180  |
| D L N D V Y V Y W Q T S E S K                                    |      |
| 50 55 60   |      |
| ACC GTG GTG ACC TAC CAC ATC CCA CAG AAC AGC TCC TTG GAA AAC      | 225  |
| T V V T Y H I P Q N S S L E N                                    |      |
| 65 70 75   |      |
| GTG GAC AGC CGC TAC CGG AAC CGA GCC CTG ATG TCA CCG GCC GGC      | 270  |
| V D S R Y R N R A L M S P A G                                    |      |
| 80 85 90   |      |
| ATG CTG CGG GGC GAC TTC TCC CTG CGC TTG TTC AAC GTC ACC CCC      | 315  |
| M L R G D F S L R L F N V T P                                    |      |
| 95 100 105   |      |
| CAG GAC GAG CAG AAG TTT CAC TGC CTG GTG TTG AGC CAA TCC CTG      | 360  |
| Q D E Q K F H C L V L S Q S L                                    |      |
| 110 115 120  |      |
| GGA TTC CAG GAG GTT TTG AGC GTT GAG GTT ACA CTG CAT GTG GCA      | 405  |
| G F Q E V L S V E V T L H V A                                    |      |
| 125 130 135  |      |
| GCA AAC TTC AGC GTG CCC GTC GTC AGC GCC CCC CAC AGC CCC TCC      | 450  |
| A N F S V P V V S A P H S P S                                    |      |
| 140 145 150  |      |
| CAG GAT GAG CTC ACC TTC ACG TGT ACA TCC ATA AAC GGC TAC CCC      | 495  |
| Q D E L T F T C T S I N G Y P                                    |      |
| 155 160 165  |      |
| AGG CCC AAC GTG TAC TGG ATC AAT AAG ACG GAC AAC AGC CTG CTG      | 540  |
| R P N V Y W I N K T D N S L L                                    |      |
| 170 175 180  |      |
| GAC CAG GCT CTG CAG AAT GAC ACC GTC TTC TTG AAC ATG CGG GGC      | 585  |
| D Q A L Q N D T V F L N M R G                                    |      |
| 185 190 195  |      |
| TTG TAT GAC GTG GTC AGC GTG CTG AGG ATC GCA CGG ACC CCC AGC      | 630  |
| L Y D V V S V L R I A R T P S                                    |      |
| 200 205 210  |      |
| GTG AAC ATT GGC TGC TGC ATA GAG AAC GTG CTT CTG CAG CAG AAC      | 675  |
| V N I G C C I E N V L L Q Q N                                    |      |
| 215 220 225  |      |

002211 02482260



Figure 1 consists of 11 bar charts, each representing a different demographic or lifestyle category. Each chart compares the percentage of respondents across seven age groups: 18-24, 25-34, 35-44, 45-54, 55-64, 65-74, and 75+. The categories are: 1. Gender, 2. Education, 3. Income, 4. Marital Status, 5. Employment, 6. Health, 7. Living Arrangement, 8. Transportation, 9. Communication, 10. Social Media, and 11. Hobbies. The data shows varying trends across age groups for each category, such as higher internet usage in younger groups and higher car ownership in middle-aged groups.

|           |             |             |            |            |             |     |
|-----------|-------------|-------------|------------|------------|-------------|-----|
| human     | MRLGSP----  | -----       | -----G     | L-LF-LLFSS | LRADTQEKEV  | 25  |
| mouse     | MQLKPCPCFVS | LGTRQPVWKK  | LHVSSGFFSG | LGLFLLLLSS | LCAASAEDEV  | 50  |
| Consensus | M.L..P....  | .....       | .....G     | L.LF.LL.SS | L.A...E.EV  | 50  |
| human     | RAMVGSDEL   | SCACPEGSRF  | DLNDVYVYQ  | TSESKTVVTY | HIPQNSSLN   | 75  |
| mouse     | GAMVGSNVVL  | SCIDPHRRHF  | NLSGLYVYQ  | IENPEVSVTY | YLPYKSPGIN  | 100 |
| Consensus | .AMVGS.V.L  | SC...P....F | .L...YVYQ  | .....VTY   | ..P..S...N  | 100 |
| human     | VDSRYNRAL   | MSPAGMLRGD  | FSLRLFNVTP | QDEQKFHCLV | LSQ-SLGFQE  | 124 |
| mouse     | VDSSYKNRGH  | LSLDSMKQGN  | FSLYLKNVTP | QDTQEFTCRV | FMNTATELVK  | 150 |
| Consensus | VDS.Y.NR..  | .S...M..G.  | FSL.L.NVTP | QD.Q.F.C.V | .....       | 150 |
| human     | VLSVEVTLHV  | AANFSVPVVS  | APHSPSQ-DE | LTFTCTSING | YPRPNVYWIN  | 173 |
| mouse     | ILEEVVRLRV  | AANFSTPVIS  | TSOSSNPGQE | RTYTCMSKNG | YPEPNLYWIN  | 200 |
| Consensus | .L...V.L.V  | AANFS.PV.S  | ...S.....E | .T.TC.S.NG | YP.PN.YWIN  | 200 |
| human     | KTDNSLLDQA  | LQNDTVFLNM  | RGLYDVVSVL | RIARTPSVNI | GCCIENVLLQ  | 223 |
| mouse     | TTDNSLIDTA  | LQNNTVYLNK  | LGLYDVISTL | RLPWTSRGDV | LCCVENVALH  | 250 |
| Consensus | .TDNSL.D.A  | LQN.TV.LN.  | .GLYDV.S.L | R...T..... | .CC.ENV.L.  | 250 |
| human     | QNLTVGSQTG  | NDIGERDKIT  | ENPVSTGEKN | AATWSILAVL | CLLVVVAVAI  | 273 |
| mouse     | QNITSISQAE  | SFTGNNTKNP  | QETHNNELKV | LV--PVLAVL | AAAASFVSFII | 298 |
| Consensus | QN.T...SQ.. | ...G...K..  | .....K.    | .....LAVL  | .....V...I  | 300 |
| human     | GWVCRDRCLQ  | HSYAGAWAVS  | PETELTGHV  |            |             | 302 |
| mouse     | YR--RTR-PH  | RSYTGPKTVQ  | LE--LTDHA  |            |             | 322 |
| Consensus | ....R.R...  | .SY.G...V.  | .E..LT.H.  |            |             | 329 |

Figure 1 consists of 12 bar charts, labeled (a) through (l), arranged in a 6x2 grid. Each chart shows the percentage of total protein in various fractions (A, B, C, D, E, F, G, H, I, J, K, L) for different cell lines and treatments. The y-axis represents the percentage of total protein, ranging from 0 to 100. The x-axis represents the fraction number. The charts show the distribution of protein across fractions for different cell lines and treatments, with some fractions showing significant changes in protein content.

|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
|--|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| AACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTAAATACGA |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     | -111 |
| CTCACTATAGGGAAAGCTGGTACGCCTGCAGGTACCGGTCCGGAATCCCCGGGTC  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     | -56  |
| GACCCACGCGTCCGTGAACACTGAACGCGAGGACTGTTAACTGTTTCTGGCAAAC  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     | -1   |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| ATG  | AAG | TCA | GGC | CTC  | TGG | TAT | TTC | TTT | CTC | TTC | TGC | TTG | CGC | ATT | 45  |      |
| M  | K   | S   | G   | L    | W   | Y   | F   | F   | L   | F   | C   | L   | R   | I   |     |      |
|  |     |     |     | 5    |     |     |     |     | 10  |     |     |     |     | 15  |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| AAA  | GTT | TTA | ACA | GGA  | GAA | ATC | AAT | GGT | TCT | GCC | AAT | TAT | GAG | ATG | 90  |      |
| K  | V   | L   | T   | *G   | *E  | I   | N   | G   | S   | A   | N   | Y   | E   | M   |     |      |
|  |     |     |     | 20   |     |     |     |     | 25  |     |     |     |     | 30  |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| TTT  | ATA | TTT | CAC | AAC  | GGA | GGT | GTA | CAA | ATT | TTA | TGC | AAA | TAT | CCT | 135 |      |
| F  | I   | F   | H   | N    | G   | G   | V   | Q   | I   | L   | C   | K   | Y   | P   |     |      |
|  |     |     |     | 35   |     |     |     |     | 40  |     |     |     |     | 45  |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| GAC  | ATT | GTC | CAG | CAA  | TTT | AAA | ATG | CAG | TTG | CTG | AAA | GGG | GGG | CAA | 180 |      |
| D  | I   | V   | Q   | Q    | F   | K   | M   | Q   | L   | L   | K   | G   | G   | Q   |     |      |
|  |     |     |     | 50   |     |     |     |     | 55  |     |     |     |     | 60  |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| ATA  | CTC | TGC | GAT | CTC  | ACT | AAG | ACA | AAA | GGA | AGT | GGA | AAC | ACA | GTG | 225 |      |
| I  | L   | C   | D   | L    | T   | K   | T   | K   | G   | S   | G   | N   | T   | V   |     |      |
|  |     |     |     | 65   |     |     |     |     | 70  |     |     |     |     | 75  |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| TCC  | ATT | AAG | AGT | CTG  | AAA | TTC | TGC | CAT | TCT | CAG | TTA | TCC | AAC | AAC | 270 |      |
| S  | I   | K   | S   | L    | K   | F   | C   | H   | S   | Q   | L   | S   | N   | N   |     |      |
|  |     |     |     | 80   |     |     |     |     | 85  |     |     |     |     | 90  |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| AGT  | GTC | TCT | TTT | TTT  | CTA | TAC | AAC | TTG | GAC | CAT | TCT | CAT | GCC | AAC | 315 |      |
| S  | V   | S   | F   | F    | L   | Y   | N   | L   | D   | H   | S   | H   | A   | N   |     |      |
|  |     |     |     | 95   |     |     |     |     | 100 |     |     |     |     | 105 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| TAT  | TAC | TTC | TGC | AAC  | CTA | TCA | ATT | TTT | GAT | CCT | CCT | CCT | TTT | AAA | 360 |      |
| Y  | Y   | F   | C   | N    | L   | S   | I   | F   | D   | P   | P   | P   | F   | K   |     |      |
|  |     |     |     | 110  |     |     |     |     | 115 |     |     |     |     | 120 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| GTA  | ACT | CTT | ACA | GGA  | GGA | TAT | TTG | CAT | ATT | TAT | GAA | TCA | CAA | CTT | 405 |      |
| V  | T   | L   | T   | G    | G   | Y   | L   | H   | I   | Y   | E   | S   | Q   | L   |     |      |
|  |     |     |     | 125  |     |     |     |     | 130 |     |     |     |     | 135 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| TGT  | TGC | CAG | CTG | AAG  | TTC | TGG | TTA | CCC | ATA | GGA | TGT | GCA | GCC | TTT | 450 |      |
| C  | C   | Q   | L   | K    | F   | W   | L   | P   | I   | G   | C   | A   | A   | F   |     |      |
|  |     |     |     | 140  |     |     |     |     | 145 |     |     |     |     | 150 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| GTT  | GTA | GTC | TGC | ATT  | TTG | GGA | TGC | ATA | CTT | ATT | TGT | TGG | CTT | ACA | 495 |      |
| V  | V   | V   | C   | I    | L   | G   | C   | I   | L   | I   | C   | W   | L   | T   |     |      |
|  |     |     |     | 155  |     |     |     |     | 160 |     |     |     |     | 165 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| AAA  | AAG | AAG | TAT | TCA  | TCC | AGT | GTG | CAC | GAC | CCT | AAC | GGT | GAA | TAC | 540 |      |
| K  | K   | K   | Y   | S    | S   | S   | V   | H   | D   | P   | N   | G   | E   | Y   |     |      |
|  |     |     |     | 170  |     |     |     |     | 175 |     |     |     |     | 180 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| ATG  | TTC | ATG | AGA | GCA  | GTG | AAC | ACA | GCC | AAA | AAA | TCT | AGA | CTC | ACA | 585 |      |
| M  | F   | M   | R   | A    | V   | N   | T   | A   | K   | K   | S   | R   | L   | T   |     |      |
|  |     |     |     | 185  |     |     |     |     | 190 |     |     |     |     | 195 |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| GAT  | GTG | ACC | CTA | TAA  |     |     |     |     |     |     |     |     |     |     | 600 |      |
| D  | V   | T   | L   | STOP |     |     |     |     |     |     |     |     |     |     |     |      |
|  |     |     |     | 199  |     |     |     |     |     |     |     |     |     |     |     |      |
|  |     |     |     |      |     |     |     |     |     |     |     |     |     |     |     |      |
| TATGGAAGCTCTGGCACCCAGGCATGAAGCACGTTGGCCAGTTTTCTCAACTTGA  |     |     |     |      |     |     |     |     |     |     |     |     |     | 655 |     |      |
| AGTGCAAGATTCTCTTATTTCCGGGACCACGGAGAGTCTGACTTAACTACATACA  |     |     |     |      |     |     |     |     |     |     |     |     |     | 710 |     |      |



Figure 13B

|       |   |     |
|-------|---|-----|
| hCRP1 | MKSGLWYFFLFCLRIKVLTGEINGSANYEMFIFHNGGVQILCKYPDIVQQ  | 50  |
| mCRP1 | .    :.       .:.                :                  |     |
| mCRP1 | MKPYFCRVFVFCFLIRLLTGEINGSADHRMFSFHNGGVQISCKYPETVQQ  | 50  |
| hCRP1 | FKMQLLKGGQILCDLTCTKGSGNTVSIKSLKFCHSQLSNNSVSFFLYNLD  | 100 |
| mCRP1 | . : :.       .    .                                 |     |
| mCRP1 | LKMRLFREREVLCELTKTKGSGNAVSIKNPMLCLYHLSNNSVSFFLNND   | 100 |
| hCRP1 | HSHANYFFCNLSIFDPPPFKV.TLTGGYLHIYESQLCCQLKFWLPIGCAA  | 149 |
| mCRP1 | .       .    .    .             :                   |     |
| mCRP1 | SSQGSYYFCSLISIFDPPPFQERNLSSGYLHIYESQLCCQLKLWLPVGCAA | 150 |
| hCRP1 | FVVVCILGCILICWLTCKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL   | 199 |
| mCRP1 | :        .  |     |
| mCRP1 | FVVVLLFGCILIIFWSKKKYSSSVHDPNSEYMFMAAVNTNKKSRLAGVTS  | 200 |



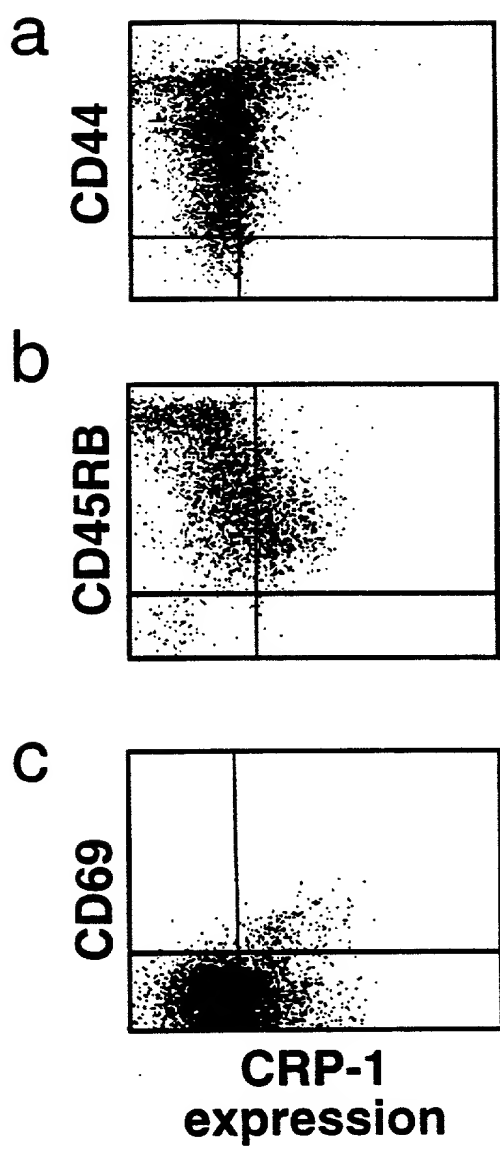
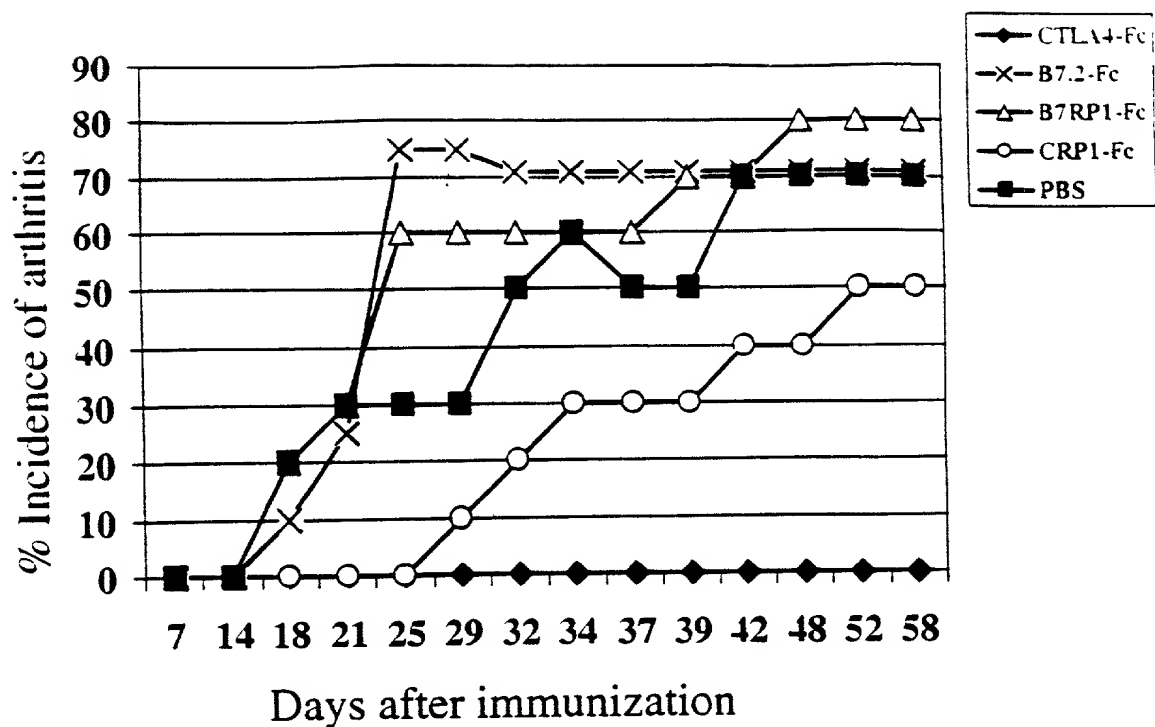


Figure 14



A.



B.

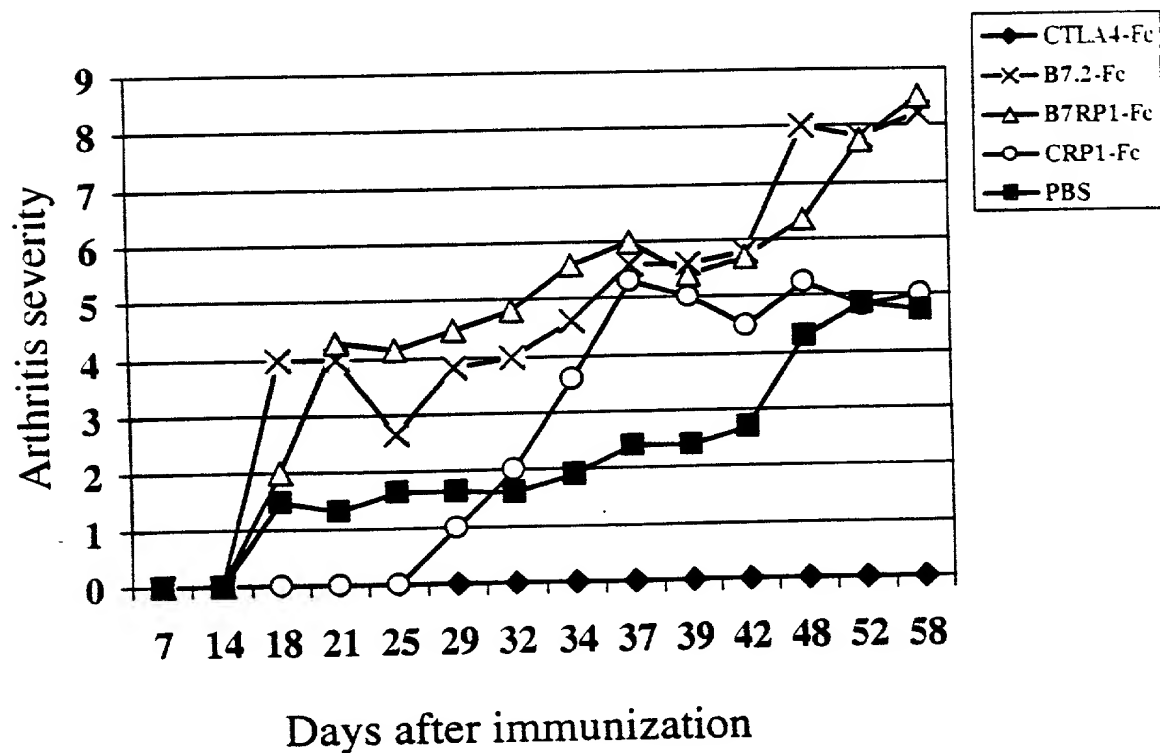
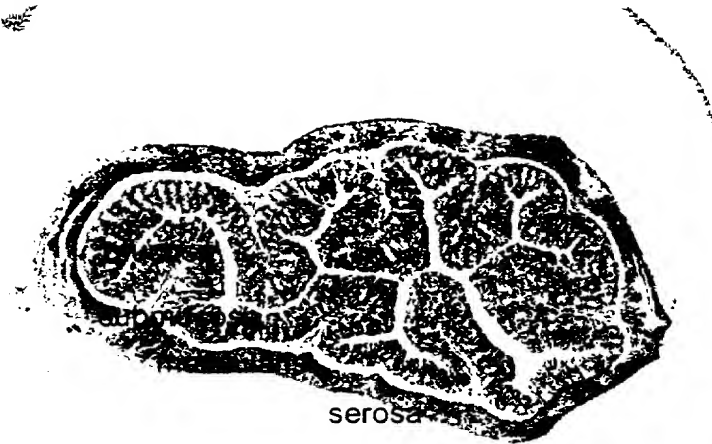


Figure 16



A. Control mouse#53F:Prox. colon 40X



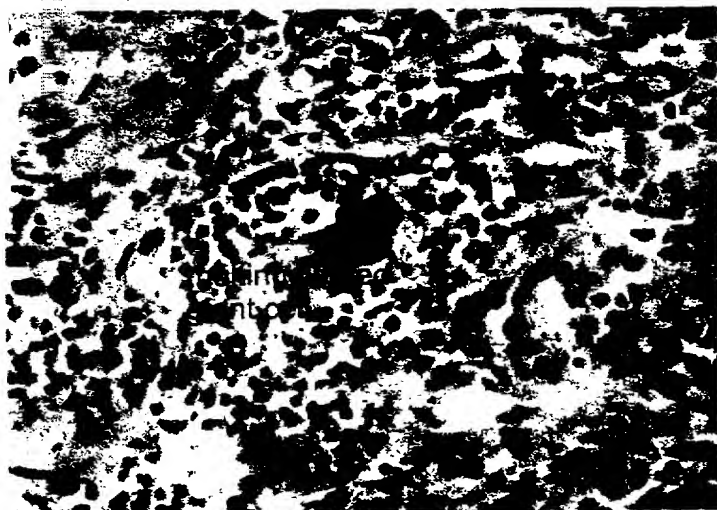
B. Mouse#111F:Prox. colon 40X



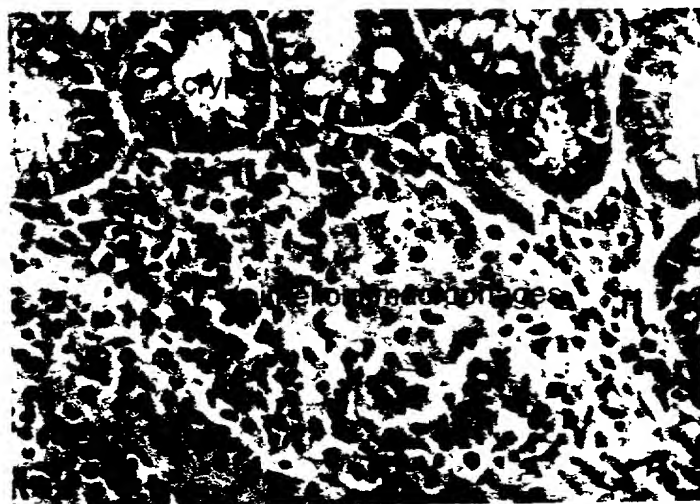
C. Mouse#111F: Prox. colon 20X



D. Mouse#111F: closeup of mucosa 100X

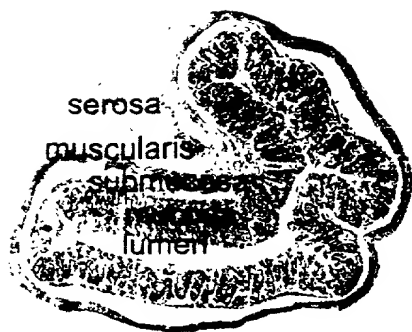


E. Mouse#112F: Giant cell, submucosa

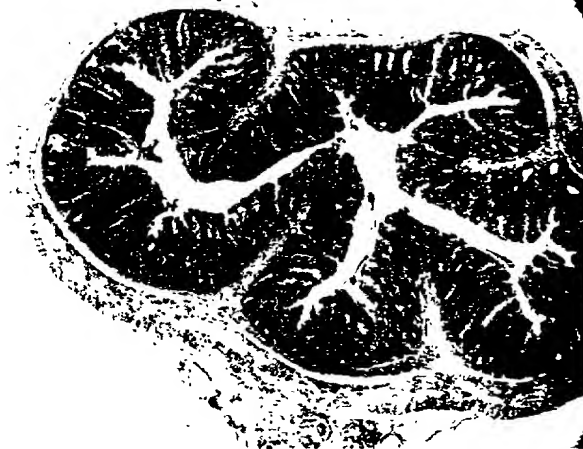


F. Mouse#112F: epithelioid macrophages

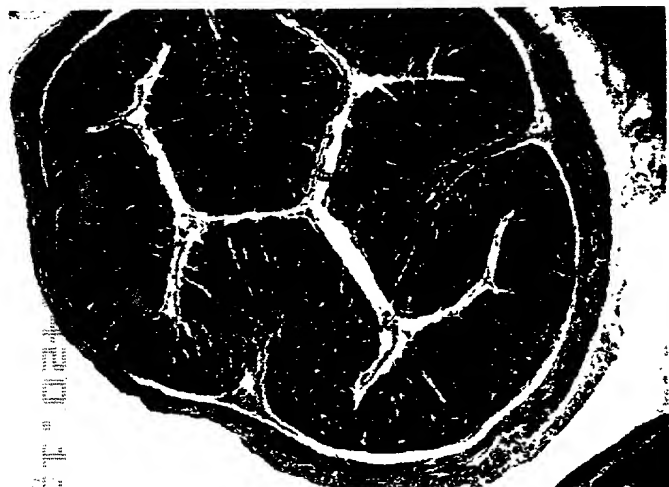
Figure 17



A. Control mouse#53F:Distal colon, 40X



B. mouse#111F:Distal colitis, 40X



C. mouse#55M:Distal colitis, 40X



D. mouse#112F:Distal colon, 40X



E. mouse#112:CD3+ T-cells, 40X



F. mouse#112:closeup, 100X

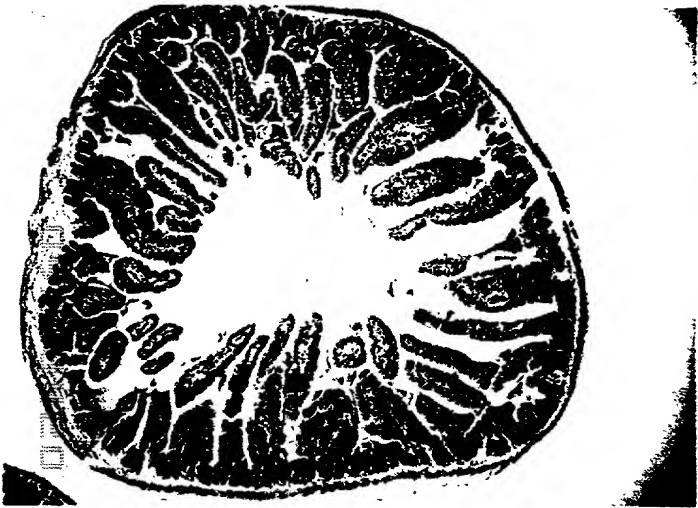
Figure 18



A. Control mouse#53F:duodenum, 40X



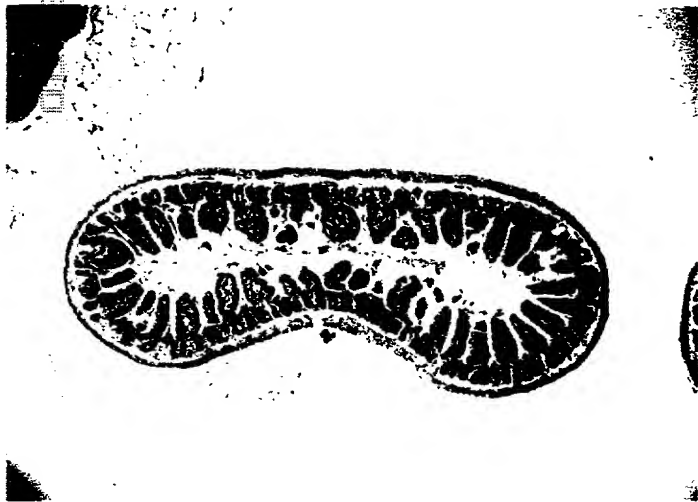
B. Mouse#51F:duodenum, 40X



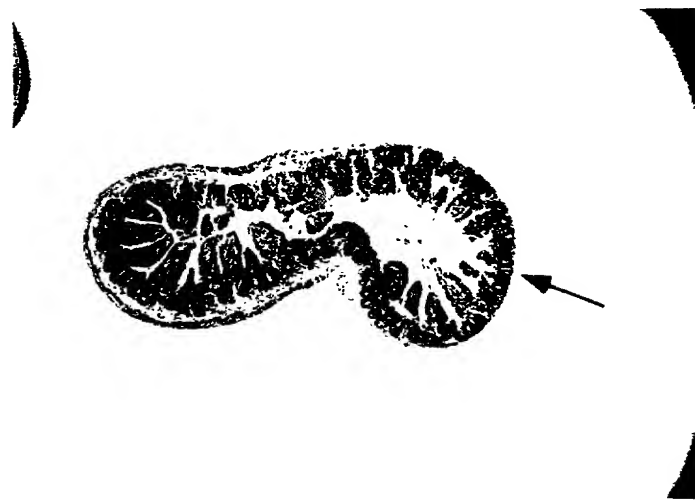
C. Control mouse#53F:jejunum, 40X



D. Mouse#51F:jejunal hyperplasia, 40X



E. Control mouse#53F:ileum, 40X



F. Mouse#231M:ileal atrophy, 40X

Figure 19

Figure 20

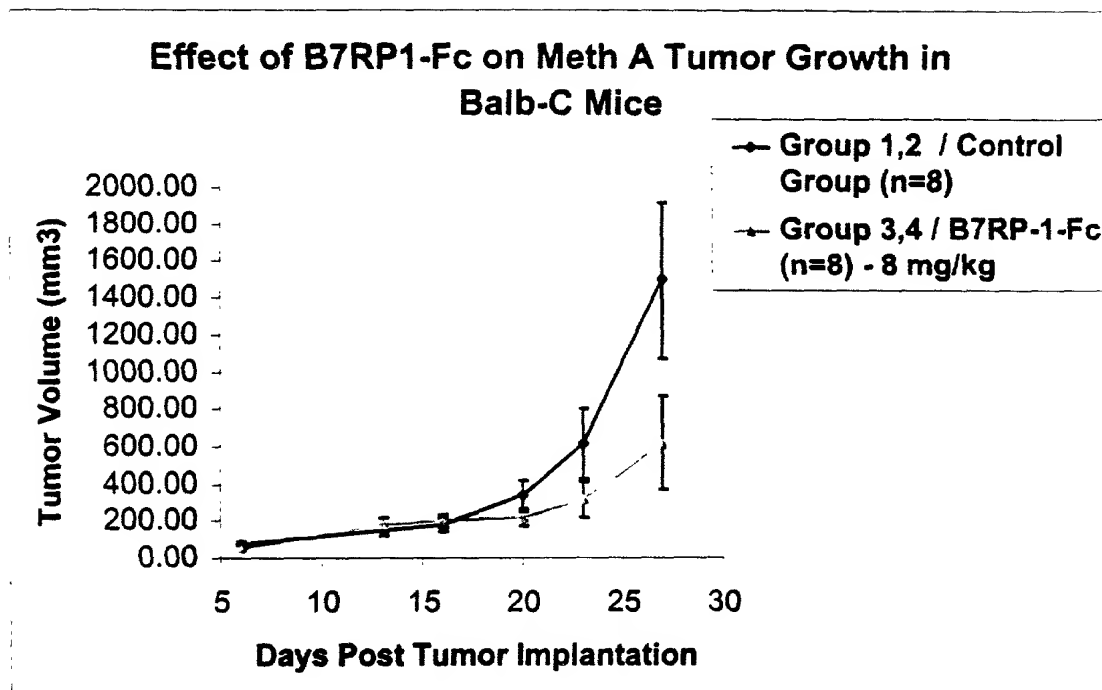


Figure 21A/B.

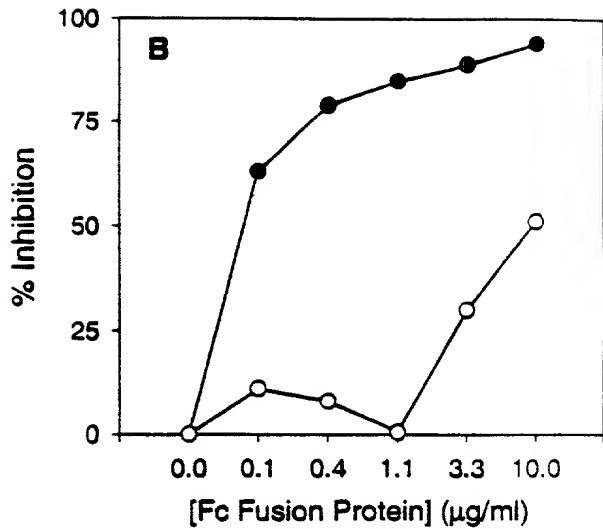
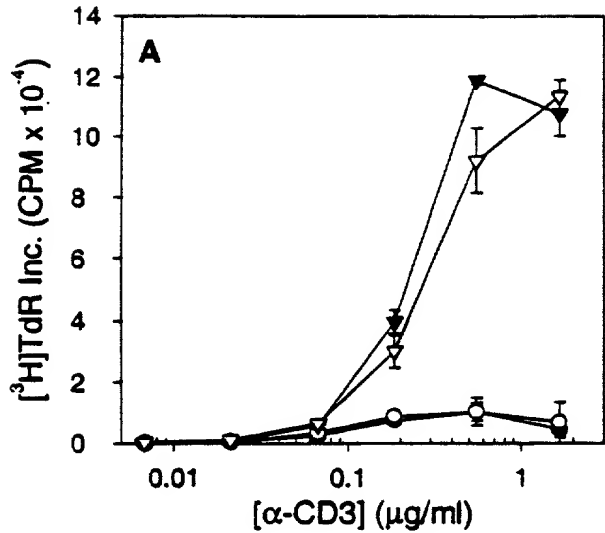




Figure 21C.

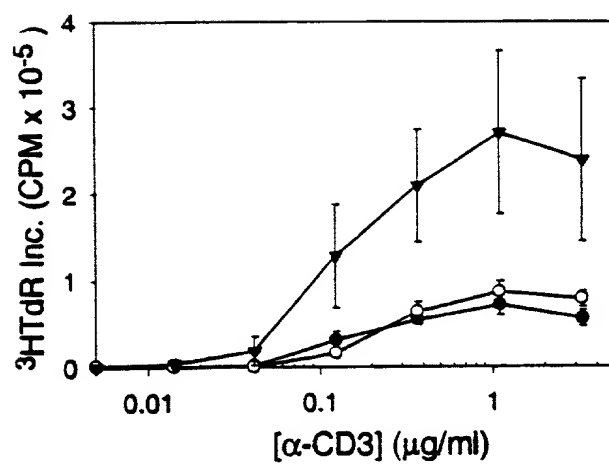


Figure 21D

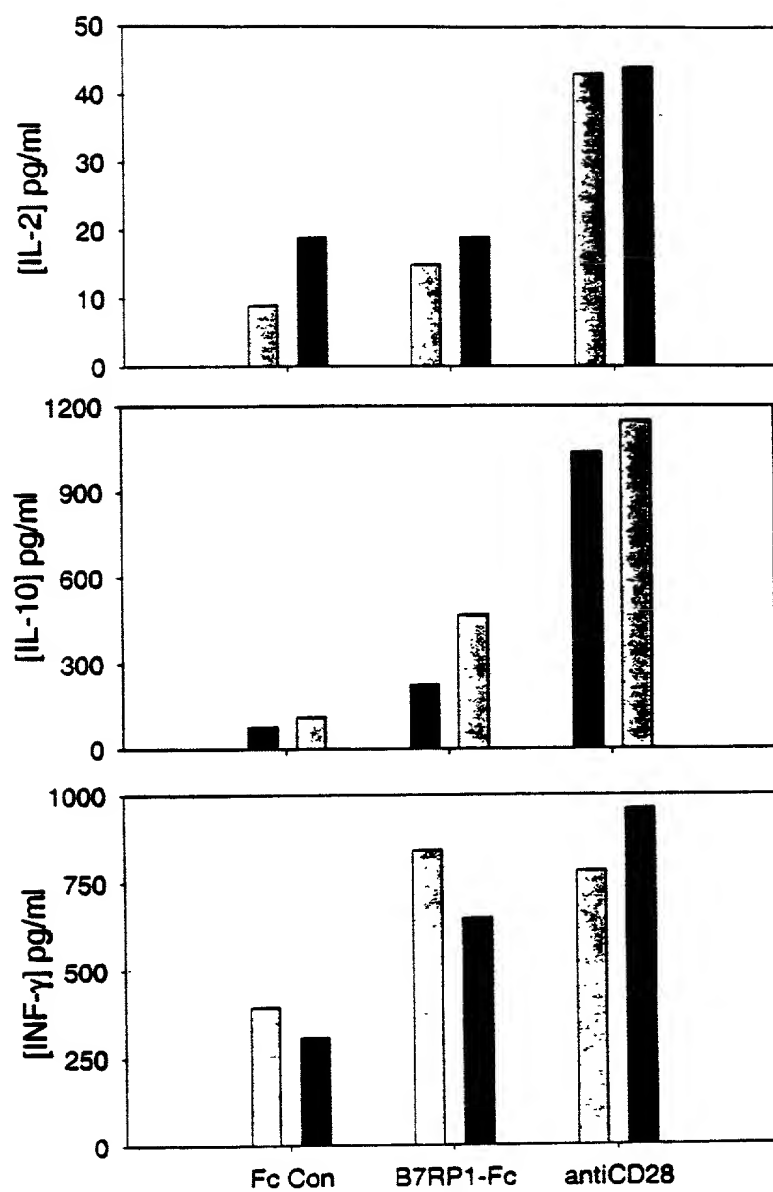


Figure 22

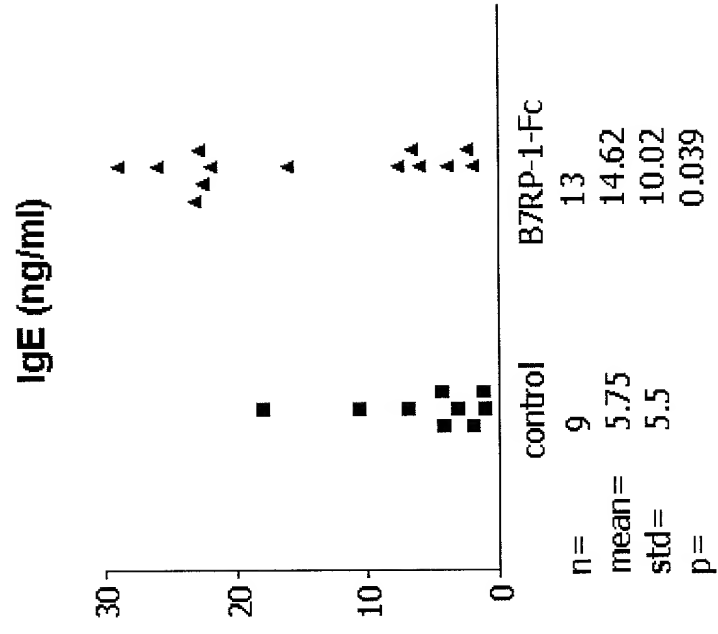
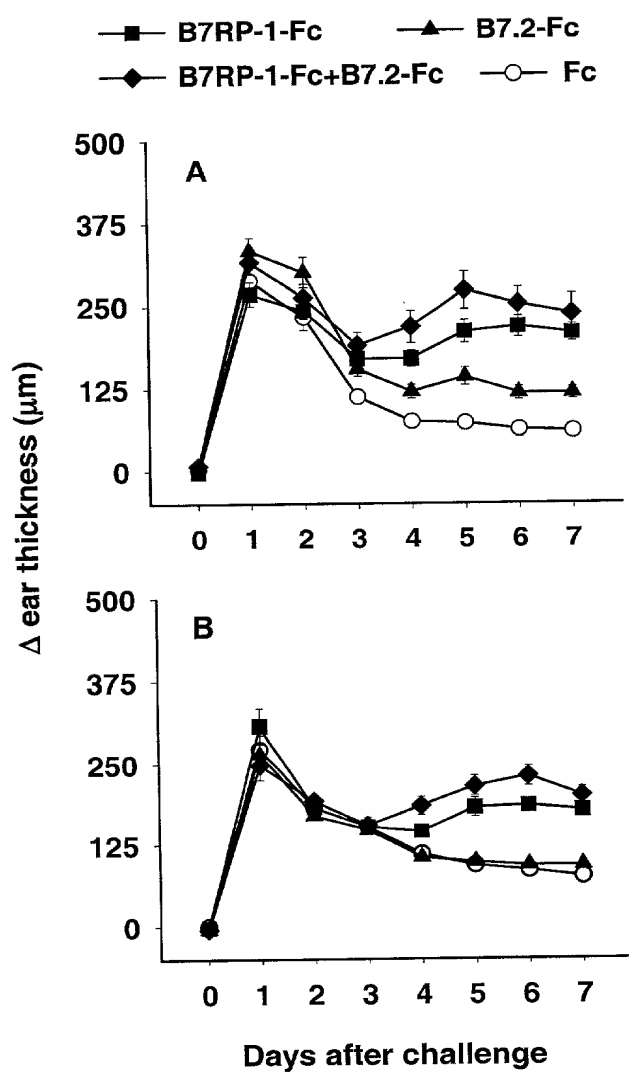


Figure 23



**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

Novel Polypeptides Involved in Immune Response

which is described and claimed in the specification which:

- ☒ is attached hereto.  
☐ was filed on \_\_\_\_\_  
as Application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

| PRIOR FOREIGN APPLICATION(S) |                    |                                   |                  |
|------------------------------|--------------------|-----------------------------------|------------------|
| Country                      | Application Number | Filing Date<br>(day, month, year) | Priority Claimed |
| PCT                          | PCT/US00/01871     | 27 January 2000                   | Yes              |
|                              |                    |                                   |                  |

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the filing date of this application:

| PRIOR U.S. APPLICATIONS |                  |         |
|-------------------------|------------------|---------|
| APPLICATION SERIAL NO.  | FILING DATE      | STATUS  |
| 09/264,527              | March 8, 1999    | Pending |
| 09/244,448              | February 3, 1999 | Pending |

**DECLARATION AND POWER OF ATTORNEY (cont'd)**

Power of Attorney: As a named inventor I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Steven M. Odre (Reg. No. 29,094), Ron K. Levy (Reg. No. 31,539), Scott N. Bernstein (Reg. No. 38,827), Joseph W. Bullock (Reg. No. 37,103), Robert R. Cook (Reg. No. 31,602), Monique L. Cordray (Reg. No. 34,802), Craig A. Crandall (Reg. No. 38,416), Daniel R. Curry (Reg. No. 32,727), Joan D. Eggert (Reg. No. 32,980), Timothy J. Gaul (Reg. No. 33,111), Matthew W. Knight (Reg. No. 36,846), Richard J. Mazza (Reg. No. 27,657), Karen L. Nicastro (Reg. No. 35,968), Nancy A. Oleski (Reg. No. 34,688), Karol M. Pessin (Reg. No. 34,899), Frank S. Ungemach (Reg. No. 34,449), Stuart L. Watt (Reg. No. 32,511), Wendy A. Whiteford (Reg. No. 36,964), Robert B. Winter (Reg. No. 34,458), Thomas D. Zindrick (Reg. No. 32,185), said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

Please send all future correspondence to:

U.S. Patent Operations/RBW  
Dept. 4300, M/S 27-4-A  
AMGEN INC.  
One Amgen Center Drive  
Thousand Oaks, California 91320-1799

Direct Telephone Calls To:

Robert B. Winter  
Attorney/Agent for Applicant(s)  
Registration No.: 34,458  
Phone: (805) 447- 2425

**DECLARATION AND POWER OF ATTORNEY (cont'd)**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole  
or First Inventor:

Steven K. Yoshinaga

Inventor's Signature:

Date:

Residence and  
Post Office Address:

1896 Calle Salto, Thousand Oaks, California, 91360, USA

(Address, City, State, Zip Code, Country)

Citizenship:

USA

Full Name of Second  
Joint Inventor, if Any:

Tak W. Mak

Inventor's Signature:

Date:

Residence and  
Post Office Address:

25 Elgin Avenue, Toronto, Ontario, M5R 1G5, Canada

(Address, City, State, Zip Code, Country)

Citizenship:

Canada

Full Name of Third  
Joint Inventor, if Any:

Arda Shahinian

Inventor's Signature:

Date:

Residence and  
Post Office Address:

85 Havelock Gate, Markham, Ontario, L3S 3P6, Canada

(Address, City, State, Zip Code, Country)

Citizenship:

Canada

Full Name of Fourth  
Joint Inventor, if Any:

Anna Tafuri Bladt

Inventor's Signature:

Date:

Residence and  
Post Office Address:

44 Gerrard Street West, Suite 1402, Toronto, Ontario, M5G 2K2, Canada

(Address, City, State, Zip Code, Country)

Citizenship:

Italy

Full Name of Fifth  
Joint Inventor, if Any:

Giorgio Senaldi

Date: \_\_\_\_\_

Date: \_\_\_\_\_

2846 White Ridge Place #24, Thousand Oaks, California, 91362, USA

(Address, City, State, Zip Code, Country)

Italy \_\_\_\_\_



&lt;120&gt; NOVEL POLYPEPTIDES INVOLVED IN IMMUNE RESPONSE

<140> 09/264,527

<141> 1999-03-08

<160> 35

&lt;170&gt; PatentIn Ver. 2.1

 $\langle 210 \rangle$  1

<211> 600

<212> DNA

<213> mouse

<220>

<221> CDS

```
<222> Complement((1)..(600))
```

<400> 1

atg aag ccg tac ttc tgc cgt gtc ttt gtc ttc tgc ttc cta atc aga 48  
Met Lys Pro Tyr Phe Cys Arg Val Phe Val Phe Cys Phe Leu Ile Arg  
1 5 10 15

ctt tta aca gga gaa atc aat ggc tgc gcc gat cat agg atg ttt tca 96  
 Leu Leu Thr Gly Glu Ile Asn Gly Ser Ala Asp His Arg Met Phe Ser  
 20 25 30

ttt cac aat gga ggt gta cag att tct tgt aaa tac cct gag act gtc 144  
Phe His Asn Gly Gly Val Gln Ile Ser Cys Lys Tyr Pro Glu Thr Val  
35 40 45

cag cag tta aaa atg cga ttg ttc aga gag aga gaa gtc ctc tgc gaa 192  
Gln Gln Leu Lys Met Arg Leu Phe Arg Glu Arg Glu Val Leu Cys Glu  
50 55 60

ctc acc aag acc aag gga agc gga aat gcg gtg tcc atc aag aat cca 240  
Leu Thr Lys Thr Lys Gly Ser Gly Asn Ala Val Ser Ile Lys Asn Pro  
65 70 75 80

atg ctc tgt cta tat cat ctg tca aac aac agc gtc tct ttt ttc cta 288  
Met Leu Cys Leu Tyr His Leu Ser Asn Asn Ser Val Ser Phe Phe Leu  
85 90 95

aac aac cca gac agc tcc cag gga agc tat tac ttc tgc agc ctg tcc 336  
Asn Asn Pro Asp Ser Ser Gln Gly Ser Tyr Tyr Phe Cys Ser Leu Ser  
100 105 110

att ttt gac cca cct cct ttt caa gaa agg aac ctt agt gga gga tat 384  
Ile Phe Asp Pro Pro Pro Phe Gln Glu Arg Asn Leu Ser Gly Gly Tyr  
115 120 125

ttg cat att tat gaa tcc cag ctc tgc tgc cag ctg aag ctc tgg cta 432  
Leu His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Leu Trp Leu  
130 135 140

ccc gta ggg tgt gca gct ttc gtt gtg gta ctc ctt ttt gga tgc ata 480  
Pro Val Gly Cys Ala Ala Phe Val Val Val Leu Leu Phe Gly Cys Ile  
145 150 155 160

```
<210> 3
<211> 200
<212> PRT
```

$\langle 400 \rangle$  3

$\langle 210 \rangle$  4

<211> 218

<212> PRT

<213> mouse

 $\langle 400 \rangle$  4

|           |           |           |           |          |           |           |           |           |           |           |           |           |           |           |           |
|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Met<br>1  | Thr       | Leu       | Arg       | Leu<br>5 | Leu       | Phe       | Leu       | Ala       | Leu<br>10 | Asn       | Phe       | Phe       | Ser       | Val<br>15 | Gln       |
| Val       | Thr       | Glu       | Asn<br>20 | Lys      | Ile       | Leu       | Val       | Lys<br>25 | Gln       | Ser       | Pro       | Leu       | Leu<br>30 | Val       | Val       |
| Asp       | Ser       | Asn<br>35 | Glu       | Val      | Ser       | Leu       | Ser<br>40 | Cys       | Arg       | Tyr       | Ser       | Tyr<br>45 | Asn       | Leu       | Leu       |
| Ala       | Lys<br>50 | Glu       | Phe       | Arg      | Ala       | Ser<br>55 | Leu       | Tyr       | Lys       | Gly       | Val<br>60 | Asn       | Ser       | Asp       | Val       |
| Glu<br>65 | Val       | Cys       | Val       | Gly      | Asn<br>70 | Gly       | Asn       | Phe       | Thr       | Tyr<br>75 | Gln       | Pro       | Gln       | Phe       | Arg<br>80 |



```
<210> 6
<211> 966
<212> DNA
<213> mouse

<220>
<221> CDS
<222> Complement((1)..(966))
```

[illegible]

```

<400> 7
Met Gln Leu Lys Cys Pro Cys Phe Val Ser Leu Gly Thr Arg Gln Pro
  1          5          10          15
Val Trp Lys Lys Leu His Val Ser Ser Gly Phe Phe Ser Gly Leu Gly
          20          25          30
Leu Phe Leu Leu Leu Leu Ser Ser Leu Cys Ala Ala Ser Ala Glu Thr
  35          40          45

```

```
<210> 8
<211> 322
<212> PRT
<213> mouse
<400> 8
```

Met Gln Leu Lys Cys Pro Cys Phe Val Ser Leu Gly Thr Arg Gln Pro  
1 5 10 15  
Val Trp Lys Lys Leu His Val Ser Ser Gly Phe Phe Ser Gly Leu Gly  
20 25 30  
Leu Phe Leu Leu Leu Leu Ser Ser Leu Cys Ala Ala Ser Ala Glu Thr  
35 40 45  
Glu Val Gly Ala Met Val Gly Ser Asn Val Val Leu Ser Cys Ile Asp  
50 55 60  
Pro His Arg Arg His Phe Asn Leu Ser Gly Leu Tyr Val Tyr Trp Gln  
65 70 75 80  
Ile Glu Asn Pro Glu Val Ser Val Thr Tyr Tyr Leu Pro Tyr Lys Ser  
85 90 95  
Pro Gly Ile Asn Val Asp Ser Ser Tyr Lys Asn Arg Gly His Leu Ser  
100 105 110  
Leu Asp Ser Met Lys Gln Gly Asn Phe Ser Leu Tyr Leu Lys Asn Val  
115 120 125  
Thr Pro Gln Asp Thr Gln Glu Phe Thr Cys Arg Val Phe Met Asn Thr  
130 135 140  
Ala Thr Glu Leu Val Lys Ile Leu Glu Glu Val Val Arg Leu Arg Val  
145 150 155 160  
Ala Ala Asn Phe Ser Thr Pro Val Ile Ser Thr Ser Asp Ser Ser Asn  
165 170 175  
Pro Gly Gln Glu Arg Thr Tyr Thr Cys Met Ser Lys Asn Gly Tyr Pro  
180 185 190  
Glu Pro Asn Leu Tyr Trp Ile Asn Thr Thr Asp Asn Ser Leu Ile Asp  
195 200 205  
Thr Ala Leu Gln Asn Asn Thr Val Tyr Leu Asn Lys Leu Gly Leu Tyr  
210 215 220  
Asp Val Ile Ser Thr Leu Arg Leu Pro Trp Thr Ser Arg Gly Asp Val  
225 230 235 240  
Leu Cys Cys Val Glu Asn Val Ala Leu His Gln Asn Ile Thr Ser Ile  
245 250 255  
Ser Gln Ala Glu Ser Phe Thr Gly Asn Asn Thr Lys Asn Pro Gln Glu  
260 265 270  
Thr His Asn Asn Glu Leu Lys Val Leu Val Pro Val Leu Ala Val Leu  
275 280 285  
Ala Ala Ala Ala Phe Val Ser Phe Ile Ile Tyr Arg Arg Thr Arg Pro  
290 295 300  
His Arg Ser Tyr Thr Gly Pro Lys Thr Val Gln Leu Glu Leu Thr Asp  
305 310 315 320  
His Ala

003417 02482460



<400> 9

Phe Leu

```
<210> 10
<211> 327
<212> PRT
<213> Artificial Sequence
```

|                   |            |            |            |            |            |            |            |            |            |            |            |            |            |            |            |
|-------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| <400><br>Met<br>1 | Xaa        | Xaa        | Xaa        | Cys<br>5   | Xaa        | Cys        | Xaa        | Xaa        | Xaa<br>10  | Leu        | Xaa        | Xaa        | Xaa        | Xaa<br>15  | Pro        |
| Xaa               | Xaa        | Xaa        | Xaa<br>20  | Leu        | Xaa        | Xaa        | Xaa        | Xaa<br>25  | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>30  | Leu        | Xaa        |
| Leu               | Phe        | Xaa<br>35  | Leu        | Leu        | Xaa        | Xaa        | Xaa<br>40  | Ser        | Xaa        | Xaa        | Xaa        | Xaa<br>45  | Xaa        | Xaa        | Xaa        |
| Xaa               | Xaa<br>50  | Xaa        | Xaa        | Xaa        | Xaa        | Val<br>55  | Xaa        | Xaa        | Xaa        | Val        | Xaa<br>60  | Leu        | Xaa        | Cys        | Xaa        |
| Xaa<br>65         | Xaa        | Xaa        | Xaa        | Xaa        | His<br>70  | Xaa        | Xaa        | Xaa        | Ser        | Xaa<br>75  | Xaa        | Xaa        | Xaa        | Tyr        | Trp<br>80  |
| Gln               | Xaa        | Xaa        | Xaa        | Xaa<br>85  | Xaa        | Val        | Xaa        | Xaa        | Xaa<br>90  | Xaa        | Xaa        | Leu        | Xaa        | Xaa<br>95  | Xaa        |
| Xaa               | Xaa        | Xaa        | Xaa<br>100 | Xaa        | Val        | Xaa        | Xaa        | Xaa<br>105 | Tyr        | Lys        | Asn        | Arg        | Xaa<br>110 | Xaa        | Xaa        |
| Xaa               | Leu        | Xaa<br>115 | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>120 | Xaa        | Xaa        | Ser        | Leu        | Xaa<br>125 | Xaa        | Xaa        | Xaa        |
| Xaa               | Xaa<br>130 | Xaa        | Xaa        | Asp        | Xaa        | Xaa<br>135 | Xaa        | Xaa        | Xaa        | Cys        | Xaa<br>140 | Val        | Xaa        | Xaa        | Xaa        |
| Xaa<br>145        | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>150 | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>155 | Xaa        | Val        | Xaa        | Leu        | Xaa<br>160 |
| Xaa               | Xaa        | Ala        | Xaa        | Phe<br>165 | Ser        | Thr        | Pro        | Xaa        | Ile<br>170 | Xaa        | Xaa        | Ser        | Xaa        | Xaa<br>175 | Xaa        |
| Xaa               | Xaa        | Xaa        | Xaa<br>180 | Xaa        | Arg        | Xaa        | Xaa        | Thr<br>185 | Cys        | Xaa        | Xaa        | Xaa        | Xaa<br>190 | Gly        | Xaa        |
| Pro               | Xaa        | Pro<br>195 | Xaa        | Xaa        | Xaa        | Trp        | Xaa<br>200 | Xaa        | Asn        | Xaa        | Xaa        | Xaa<br>205 | Xaa        | Xaa        | Xaa        |
| Ile               | Xaa<br>210 | Thr        | Xaa        | Xaa        | Xaa        | Xaa<br>215 | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>220 | Xaa        | Xaa        | Xaa        | Xaa        |
| Xaa<br>225        | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>230 | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>235 | Xaa        | Thr        | Xaa        | Xaa        | Xaa<br>240 |
| Xaa               | Xaa        | Xaa        | Xaa        | Xaa<br>245 | Xaa        | Xaa        | Xaa        | Val        | Xaa<br>250 | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>255 | Xaa        |
| Xaa               | Xaa        | Xaa        | Xaa<br>260 | Xaa        | Xaa        | Xaa        | Xaa        | Xaa<br>265 | Xaa        | Asn        | Xaa        | Xaa        | Xaa<br>270 | Xaa        | Xaa        |

```
<210> 11
<211> 864
<212> DNA
<213> mouse

<220>
<221> CDS
<222> Complement((1)..(864))
```

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> | 11  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| atg   | cgg | ctg | ggc | agt | cct | gga | ctg | ctc | ttc | ctg | ctc | ttc | agc | agc | ctt | 48  |
| Met   | Arg | Leu | Gly | Ser | Pro | Gly | Leu | Leu | Phe | Leu | Leu | Phe | Ser | Ser | Leu |     |
|       |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |     |
| cga   | gct | gat | act | cag | gag | aag | gaa | gtc | aga | gcg | atg | gta | ggc | agc | gac | 96  |
| Arg   | Ala | Asp | Thr | Gln | Glu | Lys | Glu | Val | Arg | Ala | Met | Val | Gly | Ser | Asp |     |
|       |     |     |     | 20  |     |     |     | 25  |     |     |     | 30  |     |     |     |     |
| gtg   | gag | ctc | agc | tgc | gct | tgc | cct | gaa | gga | agc | cgt | ttt | gat | tta | aat | 144 |
| Val   | Glu | Leu | Ser | Cys | Ala | Cys | Pro | Glu | Gly | Ser | Arg | Phe | Asp | Leu | Asn |     |
|       |     |     |     | 35  |     | 40  |     |     |     |     |     | 45  |     |     |     |     |
| gat   | gtt | tac | gta | tat | tgg | caa | acc | agt | gag | tcg | aaa | acc | gtg | gtg | acc | 192 |
| Asp   | Val | Tyr | Val | Tyr | Trp | Gln | Thr | Ser | Glu | Ser | Lys | Thr | Val | Val | Thr |     |
|       |     |     |     | 50  |     | 55  |     |     |     | 60  |     |     |     |     |     |     |
| tac   | cac | atc | cca | cag | aac | agc | tcc | ttg | gaa | aac | gtg | gac | agc | cgc | tac | 240 |
| Tyr   | His | Ile | Pro | Gln | Asn | Ser | Ser | Leu | Glu | Asn | Val | Asp | Ser | Arg | Tyr |     |
|       |     |     |     | 65  |     | 70  |     |     |     | 75  |     |     |     | 80  |     |     |
| cgg   | aac | cga | gcc | ctg | atg | tca | ccg | gcc | ggc | atg | ctg | cgg | ggc | gac | ttc | 288 |
| Arg   | Asn | Arg | Ala | Leu | Met | Ser | Pro | Ala | Gly | Met | Leu | Arg | Gly | Asp | Phe |     |
|       |     |     |     | 85  |     |     |     | 90  |     |     |     |     |     | 95  |     |     |
| tcc   | ctg | cgc | ttg | ttc | aac | gtc | acc | ccc | cag | gac | gag | cag | aag | ttt | cac | 336 |
| Ser   | Leu | Arg | Leu | Phe | Asn | Val | Thr | Pro | Gln | Asp | Glu | Gln | Lys | Phe | His |     |
|       |     |     |     | 100 |     |     |     | 105 |     |     |     | 110 |     |     |     |     |
| tgc   | ctg | gtg | ttg | agc | caa | tcc | ctg | gga | ttc | cag | gag | gtt | ttg | agc | gtt | 384 |
| Cys   | Leu | Val | Leu | Ser | Gln | Ser | Leu | Gly | Phe | Gln | Glu | Val | Leu | Ser | Val |     |
|       |     |     |     | 115 |     | 120 |     |     |     |     |     | 125 |     |     |     |     |
| gag   | gtt | aca | ctg | cat | gtg | gca | gca | aac | ttc | agc | gtg | ccc | gtc | gtc | agc | 432 |
| Glu   | Val | Thr | Leu | His | Val | Ala | Ala | Asn | Phe | Ser | Val | Pro | Val | Val | Ser |     |
|       |     |     |     | 130 |     | 135 |     |     |     | 140 |     |     |     |     |     |     |
| gcc   | ccc | cac | agc | ccc | tcc | cag | gat | gag | ctc | acc | ttc | acg | tgt | aca | tcc | 480 |
| Ala   | Pro | His | Ser | Pro | Ser | Gln | Asp | Glu | Leu | Thr | Phe | Thr | Cys | Thr | Ser |     |
|       |     |     |     | 145 |     | 150 |     |     |     | 155 |     |     |     | 160 |     |     |
| ata   | aac | ggc | tac | ccc | agg | ccc | aac | gtg | tac | tgg | atc | aat | aag | acg | gac | 528 |

| Ile   | Asn   | Gly | Tyr | Pro | Arg | Pro | Asn | Val | Tyr | Trp | Ile   | Asn | Lys | Thr | Asp |     |
|-------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|-----|-----|-----|
|       |       |     |     | 165 |     |     |     |     | 170 |     |       |     |     | 175 |     |     |
| aac   | agc   | ctg | ctg | gac | cag | gct | ctg | cag | aat | gac | acc   | gtc | ttc | ttg | aac | 576 |
| Asn   | Ser   | Leu | Leu | Asp | Gln | Ala | Leu | Gln | Asn | Asp | Thr   | Val | Phe | Leu | Asn |     |
|       |       |     | 180 |     |     |     |     | 185 |     |     |       |     | 190 |     |     |     |
| atg   | cgg   | ggc | ttg | tat | gac | gtg | gtc | agc | gtg | ctg | agg   | atc | gca | cgg | acc | 624 |
| Met   | Arg   | Gly | Leu | Tyr | Asp | Val | Val | Ser | Val | Leu | Arg   | Ile | Ala | Arg | Thr |     |
|       |       | 195 |     |     |     |     | 200 |     |     |     |       | 205 |     |     |     |     |
| ccc   | agc   | gtg | aac | att | ggc | tgc | tgc | ata | gag | aac | gtg   | ctt | ctg | cag | cag | 672 |
| Pro   | Ser   | Val | Asn | Ile | Gly | Cys | Cys | Ile | Glu | Asn | Val   | Leu | Leu | Gln | Gln |     |
|       | 210   |     |     |     |     | 215 |     |     |     |     | 220   |     |     |     |     |     |
| aac   | ctg   | act | gtc | ggc | agc | cag | aca | gga | aat | gac | atc   | gga | gag | aga | gac | 720 |
| Asn   | Leu   | Thr | Val | Gly | Ser | Gln | Thr | Gly | Asn | Asp | Ile   | Gly | Glu | Arg | Asp |     |
| 225   |       |     |     |     | 230 |     |     |     |     | 235 |       |     |     |     | 240 |     |
| aag   | atc   | aca | gag | aat | cca | gtc | agt | acc | ggc | gag | aaa   | aac | gcg | gcc | acg | 768 |
| Lys   | Ile   | Thr | Glu | Asn | Pro | Val | Ser | Thr | Gly | Glu | Lys   | Asn | Ala | Ala | Thr |     |
|       |       |     |     | 245 |     |     |     |     | 250 |     |       |     |     | 255 |     |     |
| tgg   | agc   | atc | ctg | gct | gtc | ctg | tgc | ctg | ctt | gtg | gtc   | gtg | gcg | gtg | gcc | 816 |
| Trp   | Ser   | Ile | Leu | Ala | Val | Leu | Cys | Leu | Leu | Val | Val   | Val | Ala | Val | Ala |     |
|       |       |     | 260 |     |     |     | 265 |     |     |     |       |     | 270 |     |     |     |
| ata   | ggc   | tgg | gtg | tgc | agg | gac | cga | tgc | ctc | caa | cac   | agc | tat | gca | ggc | 864 |
| Ile   | Gly   | Trp | Val | Cys | Arg | Asp | Arg | Cys | Leu | Gln | His   | Ser | Tyr | Ala | Gly |     |
|       |       | 275 |     |     |     |     | 280 |     |     |     |       | 285 |     |     |     |     |
| <210> | 12    |     |     |     |     |     |     |     |     |     |       |     |     |     |     |     |
| <211> | 288   |     |     |     |     |     |     |     |     |     |       |     |     |     |     |     |
| <212> | PRT   |     |     |     |     |     |     |     |     |     |       |     |     |     |     |     |
| <213> | mouse |     |     |     |     |     |     |     |     |     |       |     |     |     |     |     |
| <400> | 12    |     |     |     |     |     |     |     |     |     |       |     |     |     |     |     |
| Met   | Arg   | Leu | Gly | Ser | Pro | Gly | Leu | Leu | Phe | Leu | Leu   | Phe | Ser | Ser | Leu |     |
| 1     |       |     |     | 5   |     |     |     |     | 10  |     |       |     |     | 15  |     |     |
| Arg   | Ala   | Asp | Thr | Gln | Glu | Lys | Glu | Val | Arg | Ala | Met   | Val | Gly | Ser | Asp |     |
|       |       |     | 20  |     |     |     |     | 25  |     |     |       |     | 30  |     |     |     |
| Val   | Glu   | Leu | Ser | Cys | Ala | Cys | Pro | Glu | Gly | Ser | Arg   | Phe | Asp | Leu | Asn |     |
|       |       | 35  |     |     |     |     | 40  |     |     |     |       | 45  |     |     |     |     |
| Asp   | Val   | Tyr | Val | Tyr | Trp | Gln | Thr | Ser | Glu | Ser | Lys   | Thr | Val | Val | Thr |     |
|       |       | 50  |     |     |     | 55  |     |     |     |     | 60    |     |     |     |     |     |
| Tyr   | His   | Ile | Pro | Gln | Asn | Ser | Ser | Leu | Glu | Asn | Val   | Asp | Ser | Arg | Tyr |     |
| 65    |       |     |     | 70  |     |     |     |     |     | 75  |       |     |     |     | 80  |     |
| Arg   | Asn   | Arg | Ala | Leu | Met | Ser | Pro | Ala | Gly | Met | Leu   | Arg | Gly | Asp | Phe |     |
|       |       |     |     | 85  |     |     |     |     | 90  |     |       |     |     | 95  |     |     |
| Ser   | Leu   | Arg | Leu | Phe | Asn | Val | Thr | Pro | Gln | Asp | Glu</ |     |     |     |     |     |

|             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala         | Pro | His | Ser | Pro | Ser | Gln | Asp | Glu | Leu | Thr | Phe | Thr | Cys | Thr | Ser |
| 145         |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Ile         | Asn | Gly | Tyr | Pro | Arg | Pro | Asn | Val | Tyr | Trp | Ile | Asn | Lys | Thr | Asp |
|             |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Asn         | Ser | Leu | Leu | Asp | Gln | Ala | Leu | Gln | Asn | Asp | Thr | Val | Phe | Leu | Asn |
|             |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Met         | Arg | Gly | Leu | Tyr | Asp | Val | Val | Ser | Val | Leu | Arg | Ile | Ala | Arg | Thr |
|             |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Pro         | Ser | Val | Asn | Ile | Gly | Cys | Cys | Ile | Glu | Asn | Val | Leu | Leu | Gln | Gln |
|             | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Asn         | Leu | Thr | Val | Gly | Ser | Gln | Thr | Gly | Asn | Asp | Ile | Gly | Glu | Arg | Asp |
| 225         |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Lys         | Ile | Thr | Glu | Asn | Pro | Val | Ser | Thr | Gly | Glu | Lys | Asn | Ala | Ala | Thr |
|             |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Trp         | Ser | Ile | Leu | Ala | Val | Leu | Cys | Leu | Leu | Val | Val | Val | Ala | Val | Ala |
|             |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Ile         | Gly | Trp | Val | Cys | Arg | Asp | Arg | Cys | Leu | Gln | His | Ser | Tyr | Ala | Gly |
|             |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| <210> 13    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <211> 267   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <212> PRT   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <213> Human |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <400> 13    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu         | Lys | Glu | Val | Arg | Ala | Met | Val | Gly | Ser | Asp | Val | Glu | Leu | Ser | Cys |
| 1           |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Ala         | Cys | Pro | Glu | Gly | Ser | Arg | Phe | Asp | Leu | Asn | Asp | Val | Tyr | Val | Tyr |
|             |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Trp         | Gln | Thr | Ser | Glu | Ser | Lys | Thr | Val | Val | Thr | Tyr | His | Ile | Pro | Gln |
|             |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Asn         | Ser | Ser | Leu | Glu | Asn | Val | Asp | Ser | Arg | Tyr | Arg | Asn | Arg | Ala | Leu |
|             | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Met         | Ser | Pro | Ala | Gly | Met | Leu | Arg | Gly | Asp | Phe | Ser | Leu | Arg | Leu | Phe |
| 65          |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| Asn         | Val | Thr | Pro | Gln | Asp | Glu | Gln | Lys | Phe | His | Cys | Leu | Val | Leu | Ser |
|             |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Gln         | Ser | Leu | Gly | Phe | Gln | Glu | Val | Leu | Ser | Val | Glu | Val | Thr | Leu | His |
|             |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Val         | Ala | Ala | Asn | Phe | Ser | Val | Pro | Val | Val | Ser | Ala | Pro | His | Ser | Pro |
|             |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Ser         | Gln | Asp | Glu | Leu | Thr | Phe | Thr | Cys | Thr | Ser | Ile | Asn | Gly | Tyr | Pro |
|             | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Arg         | Pro | Asn | Val | Tyr | Trp | Ile | Asn | Lys | Thr | Asp | Asn | Ser | Leu | Leu | Asp |

```
<210> 14
<211> 276
<212> PRT
<213> mouse
```

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| <400> 14 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| Glu      | Thr | Glu | Val | Gly | Ala | Met | Val | Gly | Ser | Asn | Val | Val | Leu | Ser | Cys |  |
| 1        |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |
| Ile      | Asp | Pro | His | Arg | Arg | His | Phe | Asn | Leu | Ser | Gly | Leu | Tyr | Val | Tyr |  |
|          |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |
| Trp      | Gln | Ile | Glu | Asn | Pro | Glu | Val | Ser | Val | Thr | Tyr | Tyr | Leu | Pro | Tyr |  |
|          |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |
| Lys      | Ser | Pro | Gly | Ile | Asn | Val | Asp | Ser | Ser | Tyr | Lys | Asn | Arg | Gly | His |  |
|          | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |
| Leu      | Ser | Leu | Asp | Ser | Met | Lys | Gln | Gly | Asn | Phe | Ser | Leu | Tyr | Leu | Lys |  |
| 65       |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |
| Asn      | Val | Thr | Pro | Gln | Asp | Thr | Gln | Glu | Phe | Thr | Cys | Arg | Val | Phe | Met |  |
|          |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |
| Asn      | Thr | Ala | Thr | Glu | Leu | Val | Lys | Ile | Leu | Glu | Glu | Val | Val | Arg | Leu |  |
|          |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
| Arg      | Val | Ala | Ala | Asn | Phe | Ser | Thr | Pro | Val | Ile | Ser | Thr | Ser | Asp | Ser |  |
|          |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |
| Ser      | Asn | Pro | Gly | Gln | Glu | Arg | Thr | Tyr | Thr | Cys | Met | Ser | Lys | Asn | Gly |  |
|          | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |
| Tyr      | Pro | Glu | Pro | Asn | Leu | Tyr | Trp | Ile | Asn | Thr | Thr | Asp | Asn | Ser | Leu |  |
| 145      |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |
| Ile      | Asp | Thr | Ala | Leu | Gln | Asn | Asn | Thr | Val | Tyr | Leu | Asn | Lys | Leu | Gly |  |
|          |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |
| Leu      | Tyr | Asp | Val | Ile | Ser | Thr | Leu | Arg | Leu | Pro | Trp | Thr | Ser | Arg | Gly |  |

```
<210> 15
<211> 280
<212> PRT
<213> Artificial Sequence
```

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| <400> | 15  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| Glu   | Xaa | Glu | Val | Xaa | Ala | Met | Gly | Val | Ser | Xaa | Val | Xaa | Leu | Ser | Cys |  |
| 1     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |
| Xaa   | Xaa | Pro | Xaa | Xaa | Xaa | Xaa | Phe | Xaa | Leu | Xaa | Xaa | Xaa | Tyr | Val | Tyr |  |
|       |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |
| Trp   | Gln | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Val | Thr | Tyr | Xaa | Xaa | Pro | Xaa |  |
|       |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |
| Xaa   | Ser | Xaa | Xaa | Xaa | Asn | Val | Asp | Ser | Xaa | Tyr | Xaa | Asn | Arg | Xaa | Xaa |  |
|       | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |
| Xaa   | Ser | Xaa | Xaa | Xaa | Met | Xaa | Xaa | Gly | Xaa | Phe | Ser | Leu | Xaa | Leu | Xaa |  |
| 65    |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |
| Asn   | Val | Thr | Pro | Gln | Asp | Xaa | Gln | Xaa | Phe | Xaa | Cys | Xaa | Val | Xaa | Xaa |  |
|       |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |
| Xaa   | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Val | Xaa | Leu |  |
|       |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |
| Xaa   | Val | Ala | Ala | Asn | Phe | Ser | Xaa | Pro | Val | Xaa | Ser | Xaa | Xaa | Xaa | Ser |  |
|       |     | 115 |     |     |     |     | 120 |     |     |     | 125 |     |     |     |     |  |
| Xaa   | Xaa | Xaa | Xaa | Xaa | Glu | Xaa | Thr | Xaa | Thr | Cys | Xaa | Ser | Xaa | Asn | Gly |  |
|       |     | 130 |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |
| Tyr   | Pro | Xaa | Pro | Asn | Xaa | Tyr | Trp | Ile | Asn | Xaa | Thr | Asp | Asn | Ser | Leu |  |
| 145   |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |
| Xaa   | Asp | Xaa | Ala | Leu | Gln | Asn | Xaa | Thr | Val | Xaa | Leu | Asn | Xaa | Xaa | Gly |  |
|       |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |

[illegible]



| 80         |            |            |            |            |            |      |        |      |        | 85  |     |     |     |     | 90  |      |  |  |  |  |
|------------|------------|------------|------------|------------|------------|------|--------|------|--------|-----|-----|-----|-----|-----|-----|------|--|--|--|--|
| ctg        | cgg        | ggc        | gac        | ttc        | tcc        | ctg  | cgc    | ttg  | ttc    | aac | gtc | acc | ccc | cag | gac | 520  |  |  |  |  |
| Leu        | Arg        | Gly        | Asp        | Phe        | Ser        | Leu  | Arg    | Leu  | Phe    | Asn | Val | Thr | Pro | Gln | Asp |      |  |  |  |  |
|            |            |            | 95         |            |            |      |        | 100  |        |     |     |     | 105 |     |     |      |  |  |  |  |
| gag        | cag        | aag        | ttt        | cac        | tgc        | ctg  | gtg    | ttg  | agc    | caa | tcc | ctg | gga | ttc | cag | 568  |  |  |  |  |
| Glu        | Gln        | Lys        | Phe        | His        | Cys        | Leu  | Val    | Leu  | Ser    | Gln | Ser | Leu | Gly | Phe | Gln |      |  |  |  |  |
|            |            | 110        |            |            |            |      | 115    |      |        |     |     | 120 |     |     |     |      |  |  |  |  |
| gag        | gtt        | ttg        | agc        | gtt        | gag        | gtt  | aca    | ctg  | cat    | gtg | gca | gca | aac | ttc | agc | 616  |  |  |  |  |
| Glu        | Val        | Leu        | Ser        | Val        | Glu        | Val  | Thr    | Leu  | His    | Val | Ala | Ala | Asn | Phe | Ser |      |  |  |  |  |
|            | 125        |            |            |            |            | 130  |        |      |        |     | 135 |     |     |     |     |      |  |  |  |  |
| gtg        | ccc        | gtc        | gtc        | agc        | gcc        | ccc  | cac    | agc  | ccc    | tcc | cag | gat | gag | ctc | acc | 664  |  |  |  |  |
| Val        | Pro        | Val        | Val        | Ser        | Ala        | Pro  | His    | Ser  | Pro    | Ser | Gln | Asp | Glu | Leu | Thr |      |  |  |  |  |
|            | 140        |            |            |            | 145        |      |        |      |        | 150 |     |     |     |     | 155 |      |  |  |  |  |
| ttc        | acg        | tgt        | aca        | tcc        | ata        | aac  | ggc    | tac  | ccc    | agg | ccc | aac | gtg | tac | tgg | 712  |  |  |  |  |
| Phe        | Thr        | Cys        | Thr        | Ser        | Ile        | Asn  | Gly    | Tyr  | Pro    | Arg | Pro | Asn | Val | Tyr | Trp |      |  |  |  |  |
|            |            |            |            | 160        |            |      |        |      | 165    |     |     |     |     | 170 |     |      |  |  |  |  |
| atc        | aat        | aag        | acg        | gac        | aac        | agc  | ctg    | ctg  | gac    | cag | gct | ctg | cag | aat | gac | 760  |  |  |  |  |
| Ile        | Asn        | Lys        | Thr        | Asp        | Asn        | Ser  | Leu    | Leu  | Asp    | Gln | Ala | Leu | Gln | Asn | Asp |      |  |  |  |  |
|            |            |            | 175        |            |            |      |        | 180  |        |     |     |     | 185 |     |     |      |  |  |  |  |
| acc        | gtc        | ttc        | ttg        | aac        | atg        | cgg  | ggc    | ttg  | tat    | gac | gtg | gtc | agc | gtg | ctg | 808  |  |  |  |  |
| Thr        | Val        | Phe        | Leu        | Asn        | Met        | Arg  | Gly    | Leu  | Tyr    | Asp | Val | Val | Ser | Val | Leu |      |  |  |  |  |
|            |            | 190        |            |            |            |      | 195    |      |        |     |     | 200 |     |     |     |      |  |  |  |  |
| agg        | atc        | gca        | cgg        | acc        | ccc        | agc  | gtg    | aac  | att    | ggc | tgc | tgc | ata | gag | aac | 856  |  |  |  |  |
| Arg        | Ile        | Ala        | Arg        | Thr        | Pro        | Ser  | Val    | Asn  | Ile    | Gly | Cys | Cys | Ile | Glu | Asn |      |  |  |  |  |
|            | 205        |            |            |            |            | 210  |        |      |        |     | 215 |     |     |     |     |      |  |  |  |  |
| gtg        | ctt        | ctg        | cag        | cag        | aac        | ctg  | act    | gtc  | ggc    | agc | cag | aca | gga | aat | gac | 904  |  |  |  |  |
| Val        | Leu        | Leu        | Gln        | Gln        | Asn        | Leu  | Thr    | Val  | Gly    | Ser | Gln | Thr | Gly | Asn | Asp |      |  |  |  |  |
|            | 220        |            |            |            | 225        |      |        |      |        | 230 |     |     |     |     | 235 |      |  |  |  |  |
| atc        | gga        | gag        | aga        | gac        | aag        | atc  | aca    | gag  | aat    | cca | gtc | agt | acc | ggc | gag | 952  |  |  |  |  |
| Ile        | Gly        | Glu        | Arg        | Asp        | Lys        | Ile  | Thr    | Glu  | Asn    | Pro | Val | Ser | Thr | Gly | Glu |      |  |  |  |  |
|            |            |            |            | 240        |            |      |        |      | 245    |     |     |     |     | 250 |     |      |  |  |  |  |
| aaa        | aac        | gcg        | gcc        | acg        | tgg        | agc  | atc    | ctg  | gct    | gtc | ctg | tgc | ctg | ctt | gtg | 1000 |  |  |  |  |
| Lys        | Asn        | Ala        | Ala        | Thr        | Trp        | Ser  | Ile    | Leu  | Ala    | Val | Leu | Cys | Leu | Leu | Val |      |  |  |  |  |
|            |            |            | 255        |            |            |      |        | 260  |        |     |     |     | 265 |     |     |      |  |  |  |  |
| gtc        | gtg        | gcg        | gtg        | gcc        | ata        | ggc  | tgg    | gtg  | tgc    | agg | gac | cga | tgc | ctc | caa | 1048 |  |  |  |  |
| Val        | Val        | Ala        | Val        | Ala        | Ile        | Gly  | Trp    | Val  | Cys    | Arg | Asp | Arg | Cys | Leu | Gln |      |  |  |  |  |
|            |            | 270        |            |            |            |      | 275    |      |        |     |     | 280 |     |     |     |      |  |  |  |  |
| cac        | agc        | tat        | gca        | ggt        | gcc        | tgg  | gct    | gtg  | agt    | ccg | gag | aca | gag | ctc | act | 1096 |  |  |  |  |
| His        | Ser        | Tyr        | Ala        | Gly        | Ala        | Trp  | Ala    | Val  | Ser    | Pro | Glu | Thr | Glu | Leu | Thr |      |  |  |  |  |
|            | 285        |            |            |            |            | 290  |        |      |        |     | 295 |     |     |     |     |      |  |  |  |  |
| ggc        | cac        | gtt        | tgaccg     | gagc       | tcaccg     | ccca | gagcgt | ggac | agggct | tcg |     |     |     |     |     | 1145 |  |  |  |  |
| Gly        | His        | Val        |            |            |            |      |        |      |        |     |     |     |     |     |     |      |  |  |  |  |
|            | 300        |            |            |            |            |      |        |      |        |     |     |     |     |     |     |      |  |  |  |  |
| tgagacgcca | ccgtgagagg | ccaggtggca | gcttgagcat | ggactcccag | actgcagggg |      |        |      |        |     |     |     |     |     |     | 1205 |  |  |  |  |
| agcacttggg | gcagccccca | gaaggaccac | tgctggatcc | cagggagaac | ctgctggcgt |      |        |      |        |     |     |     |     |     |     | 1265 |  |  |  |  |
| tggtgtgat  | cctggaatga | ggccctttc  |            |            |            |      |        |      |        |     |     |     |     |     |     | 1294 |  |  |  |  |

002210 02022200

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| <400> | 17  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Met   | Arg | Leu | Gly | Ser | Pro | Gly | Leu | Leu | Phe | Leu | Leu | Phe | Ser | Ser | Leu |  |  |
| 1     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |  |
| Arg   | Ala | Asp | Thr | Gln | Glu | Lys | Glu | Val | Arg | Ala | Met | Val | Gly | Ser | Asp |  |  |
|       |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |  |
| Val   | Glu | Leu | Ser | Cys | Ala | Cys | Pro | Glu | Gly | Ser | Arg | Phe | Asp | Leu | Asn |  |  |
|       |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |  |
| Asp   | Val | Tyr | Val | Tyr | Trp | Gln | Thr | Ser | Glu | Ser | Lys | Thr | Val | Val | Thr |  |  |
|       | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |  |
| Tyr   | His | Ile | Pro | Gln | Asn | Ser | Ser | Leu | Glu | Asn | Val | Asp | Ser | Arg | Tyr |  |  |
| 65    |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |  |
| Arg   | Asn | Arg | Ala | Leu | Met | Ser | Pro | Ala | Gly | Met | Leu | Arg | Gly | Asp | Phe |  |  |
|       |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |  |
| Ser   | Leu | Arg | Leu | Phe | Asn | Val | Thr | Pro | Gln | Asp | Glu | Gln | Lys | Phe | His |  |  |
|       |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |  |
| Cys   | Leu | Val | Leu | Ser | Gln | Ser | Leu | Gly | Phe | Gln | Glu | Val | Leu | Ser | Val |  |  |
|       |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |
| Glu   | Val | Thr | Leu | His | Val | Ala | Ala | Asn | Phe | Ser | Val | Pro | Val | Val | Ser |  |  |
|       | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |
| Ala   | Pro | His | Ser | Pro | Ser | Gln | Asp | Glu | Leu | Thr | Phe | Thr | Cys | Thr | Ser |  |  |
| 145   |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |
| Ile   | Asn | Gly | Tyr | Pro | Arg | Pro | Asn | Val | Tyr | Trp | Ile | Asn | Lys | Thr | Asp |  |  |
|       |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |
| Asn   | Ser | Leu | Leu | Asp | Gln | Ala | Leu | Gln | Asn | Asp | Thr | Val | Phe | Leu | Asn |  |  |
|       |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |
| Met   | Arg | Gly | Leu | Tyr | Asp | Val | Val | Ser | Val | Leu | Arg | Ile | Ala | Arg | Thr |  |  |
|       |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |
| Pro   | Ser | Val | Asn | Ile | Gly | Cys | Cys | Ile | Glu | Asn | Val | Leu | Leu | Gln | Gln |  |  |
|       | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |
| Asn   | Leu | Thr | Val | Gly | Ser | Gln | Thr | Gly | Asn | Asp | Ile | Gly | Glu | Arg | Asp |  |  |
| 225   |     |     |     |     | 230 |     |     |     | 235 |     |     |     |     |     | 240 |  |  |
| Lys   | Ile | Thr | Glu | Asn | Pro | Val | Ser | Thr | Gly | Glu | Lys | Asn | Ala | Ala | Thr |  |  |
|       |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |
| Trp   | Ser | Ile | Leu | Ala | Val | Leu | Cys | Leu | Leu | Val | Val | Val | Ala | Val | Ala |  |  |
|       |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |
| Ile   | Gly | Trp | Val | Cys | Arg | Asp | Arg | Cys | Leu | Gln | His | Ser | Tyr | Ala | Gly |  |  |
|       |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |
| Ala   | Trp | Ala | Val | Ser | Pro | Glu | Thr | Glu | Leu | Thr | Gly | His | Val |     |     |  |  |
|       | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 18 | Met | Arg | Leu | Gly | Ser | Pro | Gly | Leu | Leu | Phe | Leu | Leu | Phe | Ser | Ser | Leu |
| 1        |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Arg      | Ala | Asp | Thr | Gln | Glu | Lys | Glu | Val | Arg | Ala | Met | Val | Gly | Ser | Asp |     |
|          |     |     | 20  |     |     |     |     | 25  |     |     |     |     |     | 30  |     |     |
| Val      | Glu | Leu | Ser | Cys | Ala | Cys | Pro | Glu | Gly | Ser | Arg | Phe | Asp | Leu | Asn |     |
|          |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| Asp      | Val | Tyr | Val | Tyr | Trp | Gln | Thr | Ser | Glu | Ser | Lys | Thr | Val | Val | Thr |     |
|          | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |
| Tyr      | His | Ile | Pro | Gln | Asn | Ser | Ser | Leu | Glu | Asn | Val | Asp | Ser | Arg | Tyr |     |
| 65       |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |     |
| Arg      | Asn | Arg | Ala | Leu | Met | Ser | Pro | Ala | Gly | Met | Leu | Arg | Gly | Asp | Phe |     |
|          |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
| Ser      | Leu | Arg | Leu | Phe | Asn | Val | Thr | Pro | Gln | Asp | Glu | Gln | Lys | Phe | His |     |
|          |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| Cys      | Leu | Val | Leu | Ser | Gln | Ser | Leu | Gly | Phe | Gln | Glu | Val | Leu | Ser | Val |     |
|          |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
| Glu      | Val | Thr | Leu | His | Val | Ala | Ala | Asn | Phe | Ser | Val | Pro | Val | Val | Ser |     |
|          | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |
| Ala      | Pro | His | Ser | Pro | Ser | Gln | Asp | Glu | Leu | Thr | Phe | Thr | Cys | Thr | Ser |     |
| 145      |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
| Ile      | Asn | Gly | Tyr | Pro | Arg | Pro | Asn | Val | Tyr | Trp | Ile | Asn | Lys | Thr | Asp |     |
|          |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
| Asn      | Ser | Leu | Leu | Asp | Gln | Ala | Leu | Gln | Asn | Asp | Thr | Val | Phe | Leu | Asn |     |
|          |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
| Met      | Arg | Gly | Leu | Tyr | Asp | Val | Val | Ser | Val | Leu | Arg | Ile | Ala | Arg | Thr |     |
|          |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
| Pro      | Ser | Val | Asn | Ile | Gly | Cys | Cys | Ile | Glu | Asn | Val | Leu | Leu | Gln | Gln |     |
|          | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
| Asn      | Leu | Thr | Val | Gly | Ser | Gln | Thr | Gly | Asn | Asp | Ile | Gly | Glu | Arg | Asp |     |
| 225      |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |
| Lys      | Ile | Thr | Glu | Asn | Pro | Val | Ser | Thr | Gly | Glu | Lys | Asn | Ala | Ala | Thr |     |
|          |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |     |
| Trp      | Ser | Ile | Leu | Ala | Val | Leu | Cys | Leu | Leu | Val | Val | Val | Ala | Val | Ala |     |
|          |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |     |
| Ile      | Gly | Trp | Val | Cys | Arg | Asp | Arg | Cys | Leu | Gln | His | Ser | Tyr | Ala | Gly |     |
|          |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |     |
| Ala      | Trp | Ala | Val | Ser | Pro | Glu | Thr | Glu | Leu | Thr | Gly | His | Val |     |     |     |
|          | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |     |

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|
| <400> | 19  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
| Met   | Gln | Leu | Lys | Cys | Pro | Cys | Phe | Val | Ser | Leu | Gly | Thr | Arg | Gln | Pro |  |  |  |  |
| 1     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |  |  |  |
| Val   | Trp | Lys | Lys | Leu | His | Val | Ser | Ser | Gly | Phe | Phe | Ser | Gly | Leu | Gly |  |  |  |  |
|       |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |  |  |  |
| Leu   | Phe | Leu | Leu | Leu | Leu | Ser | Ser | Leu | Cys | Ala | Ala | Ser | Ala | Glu | Thr |  |  |  |  |
|       |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |  |  |  |
| Glu   | Val | Gly | Ala | Met | Val | Gly | Ser | Asn | Val | Val | Leu | Ser | Cys | Ile | Asp |  |  |  |  |
|       | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |  |  |  |
| Pro   | His | Arg | Arg | His | Phe | Asn | Leu | Ser | Gly | Leu | Tyr | Val | Tyr | Trp | Gln |  |  |  |  |
| 65    |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |  |  |  |
| Ile   | Glu | Asn | Pro | Glu | Val | Ser | Val | Thr | Tyr | Tyr | Leu | Pro | Tyr | Lys | Ser |  |  |  |  |
|       |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |  |  |  |
| Pro   | Gly | Ile | Asn | Val | Asp | Ser | Ser | Tyr | Lys | Asn | Arg | Gly | His | Leu | Ser |  |  |  |  |
|       |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |  |  |  |
| Leu   | Asp | Ser | Met | Lys | Gln | Gly | Asn | Phe | Ser | Leu | Tyr | Leu | Lys | Asn | Val |  |  |  |  |
|       |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |  |  |
| Thr   | Pro | Gln | Asp | Thr | Gln | Glu | Phe | Thr | Cys | Arg | Val | Phe | Met | Asn | Thr |  |  |  |  |
|       | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |  |
| Ala   | Thr | Glu | Leu | Val | Lys | Ile | Leu | Glu | Glu | Val | Val | Arg | Leu | Arg | Val |  |  |  |  |
| 145   |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |  |  |
| Ala   | Ala | Asn | Phe | Ser | Thr | Pro | Val | Ile | Ser | Thr | Ser | Asp | Ser | Ser | Asn |  |  |  |  |
|       |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |  |  |
| Pro   | Gly | Gln | Glu | Arg | Thr | Tyr | Thr | Cys | Met | Ser | Lys | Asn | Gly | Tyr | Pro |  |  |  |  |
|       |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |  |  |
| Glu   | Pro | Asn | Leu | Tyr | Trp | Ile | Asn | Thr | Thr | Asp | Asn | Ser | Leu | Ile | Asp |  |  |  |  |
|       |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |  |
| Thr   | Ala | Leu | Gln | Asn | Asn | Thr | Val | Tyr | Leu | Asn | Lys | Leu | Gly | Leu | Tyr |  |  |  |  |
|       | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |  |  |
| Asp   | Val | Ile | Ser | Thr | Leu | Arg | Leu | Pro | Trp | Thr | Ser | Arg | Gly | Asp | Val |  |  |  |  |
| 225   |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |  |  |
| Leu   | Cys | Cys | Val | Glu | Asn | Val | Ala | Leu | His | Gln | Asn | Ile | Thr | Ser | Ile |  |  |  |  |
|       |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |  |  |
| Ser   | Gln | Ala | Glu | Ser | Phe | Thr | Gly | Asn | Asn | Thr | Lys | Asn | Pro | Gln | Glu |  |  |  |  |
|       |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |  |  |
| Thr   | His | Asn | Asn | Glu | Leu | Lys | Val | Leu | Val | Pro | Val | Leu | Ala | Val | Leu |  |  |  |  |
|       |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |  |
| Ala   | Ala | Ala | Ala | Phe | Val | Ser | Phe | Ile | Ile | Tyr | Arg | Arg | Thr | Arg | Pro |  |  |  |  |

His Ala

<220>  
<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

|       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> | 20  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Met   | Xaa | Leu | Xaa | Xaa | Pro | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa |
| 1     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Xaa   | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Gly | Leu | Xaa |
|       |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Leu   | Phe | Xaa | Leu | Leu | Xaa | Ser | Ser | Leu | Xaa | Ala | Xaa | Xaa | Xaa | Glu | Xaa |
|       |     | 35  |     |     |     | 40  |     |     |     |     |     | 45  |     |     |     |
| Glu   | Val | Xaa | Ala | Met | Val | Gly | Ser | Xaa | Val | Xaa | Leu | Ser | Cys | Xaa | Xaa |
|       | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Pro   | Xaa | Xaa | Xaa | Xaa | Phe | Xaa | Leu | Xaa | Xaa | Xaa | Tyr | Val | Tyr | Trp | Gln |
| 65    |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| Xaa   | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Val | Thr | Tyr | Xaa | Xaa | Pro | Xaa | Xaa | Ser |
|       |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Xaa   | Xaa | Xaa | Asn | Val | Asp | Ser | Xaa | Tyr | Xaa | Asn | Arg | Xaa | Xaa | Xaa | Ser |
|       |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Xaa   | Xaa | Xaa | Met | Xaa | Xaa | Gly | Xaa | Phe | Ser | Leu | Xaa | Leu | Xaa | Asn | Val |
|       |     | 115 |     |     |     | 120 |     |     |     |     |     | 125 |     |     |     |
| Thr   | Pro | Gln | Asp | Xaa | Gln | Xaa | Phe | Xaa | Cys | Xaa | Val | Xaa | Xaa | Xaa | Xaa |
|       | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Xaa   | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Val | Xaa | Leu | Xaa | Val |
| 145   |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Ala   | Ala | Asn | Phe | Ser | Xaa | Pro | Val | Xaa | Ser | Xaa | Xaa | Xaa | Ser | Xaa | Xaa |
|       |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Xaa   | Xaa | Xaa | Glu | Xaa | Thr | Xaa | Thr | Cys | Xaa | Ser | Xaa | Asn | Gly | Tyr | Pro |
|       |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Xaa   | Pro | Asn | Xaa | Tyr | Trp | Ile | Asn | Xaa | Thr | Asp | Asn | Ser | Leu | Xaa | Asp |
|       |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Xaa   | Ala | Leu | Gln | Asn | Xaa | Thr | Val | Xaa | Leu | Asn | Xaa | Xaa | Gly | Leu | Tyr |
|       | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Asp   | Val | Xaa | Ser | Xaa | Leu | Arg | Xaa | Xaa | Xaa | Thr | Xaa | Xaa | Xaa | Xaa | Xaa |
| 225   |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Xaa   | Cys | Cys | Xaa | Glu | Asn | Val | Xaa | Leu | Xaa | Gln | Asn | Xaa | Thr | Xaa | Xaa |

```
<210> 21
<211> 1370
<212> DNA
<213> Human

<220>
<221> 5'UTR
<222> (1)..(165)
```

```
<220>
<221> 5'UTR
<222> (1)..(165)

<220>
<221> CDS
<222> (166)..(762)
```

|             |             |            |             |             |            |     |     |     |     |     |     |     |     |     |     |     |     |
|-------------|-------------|------------|-------------|-------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 21    |             |            |             |             |            |     |     |     |     |     |     |     |     |     |     |     | 60  |
| aacaattttca | cacaggaaac  | agctatgacc | atgattacgc  | caagctctaa  | tacgactcac |     |     |     |     |     |     |     |     |     |     |     |     |
| tataggggaaa | gctgggtacgc | ctgcaggtac | cgggtccggaa | ttcccggggtc | gacccacgcg |     |     |     |     |     |     |     |     |     |     |     | 120 |
| tccgtgaaca  | ctgaacgcga  | ggactgttaa | ctgtttctgg  | caaac       | atg        | aag | tca | ggc |     |     |     |     |     |     |     |     | 177 |
|             |             |            |             |             |            |     |     |     | Met | Lys | Ser | Gly |     |     |     |     |     |
|             |             |            |             |             |            |     |     |     | 1   |     |     |     |     |     |     |     |     |
| ctc         | tggt        | tatt       | ttc         | ttt         | ctc        | ttc | tgc | ttg | cgc | att | aaa | gtt | tta | aca | gga | 225 |     |
| Leu         | Trp         | Tyr        | Phe         | Phe         | Leu        | Phe | Cys | Leu | Arg | Ile | Lys | Val | Leu | Thr | Gly |     |     |
| 5           |             |            |             |             |            |     |     |     |     |     | 15  |     |     |     |     | 20  |     |
| gaa         | atc         | aatt       | ggt         | tct         | gcc        | aat | tat | gag | atg | ttt | ata | ttt | cac | aac | gga | 273 |     |
| Glu         | Ile         | Asn        | Gly         | Ser         | Ala        | Asn | Tyr | Glu | Met | Phe | Ile | Phe | His | Asn | Gly |     |     |
|             |             |            |             | 25          |            |     |     |     |     |     |     |     |     |     | 35  |     |     |
| ggt         | gta         | caa        | att         | tta         | tgc        | aaa | tat | cct | gac | att | gtc | cag | caa | ttt | aaa | 321 |     |
| Gly         | Val         | Gln        | Ile         | Leu         | Cys        | Lys | Tyr | Pro | Asp | Ile | Val | Gln | Gln | Phe | Lys |     |     |
|             |             |            | 40          |             |            |     |     |     |     |     |     |     |     | 50  |     |     |     |
| atg         | cag         | ttg        | ctg         | aaa         | ggg        | ggg | caa | ata | ctc | tgc | gat | ctc | act | aag | aca | 369 |     |
| Met         | Gln         | Leu        | Leu         | Lys         | Gly        | Gly | Gln | Ile | Leu | Cys | Asp | Leu | Thr | Lys | Thr |     |     |
|             |             | 55         |             |             |            |     |     |     |     |     |     |     | 65  |     |     |     |     |
| aaa         | gga         | agt        | gga         | aac         | aca        | gtg | tcc | att | aag | agt | ctg | aaa | ttc | tgc | cat | 417 |     |
| Lys         | Gly         | Ser        | Gly         | Asn         | Thr        | Val | Ser | Ile | Lys | Ser | Leu | Lys | Phe | Cys | His |     |     |
|             |             | 70         |             |             |            |     |     |     |     |     |     |     | 80  |     |     |     |     |
| tct         | cag         | tta        | tcc         | aac         | aac        | agt | gtc | tct | ttt | ttt | cta | tac | aac | ttg | gac | 465 |     |
| Ser         | Gln         | Leu        | Ser         | Asn         | Asn        | Ser | Val | Ser | Phe | Phe | Leu | Tyr | Asn | Leu | Asp |     |     |
| 85          |             |            |             |             |            |     |     |     |     |     | 95  |     |     |     |     | 100 |     |

|       |       |
|-------|-------|
| <210> | 22    |
| <211> | 199   |
| <212> | PRT   |
| <213> | Human |

<400> 22  
Met Lys Ser Gly Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys  
1 5 10 15  
Val Leu Thr Gly Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile  
20 25 30  
Phe His Asn Gly Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val  
35 40 45  
Gln Gln Phe Lys Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp

```

<210> 23
<211> 199
<212> PRT
<213> Human

<400> 23
Met Lys Ser Gly Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys
  1          5          10
Val Leu Thr Gly Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile
          20          25          30
Phe His Asn Gly Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val
          35          40
Gln Gln Phe Lys Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp
          50          55          60
Leu Thr Lys Thr Lys Gly Ser Gly Asn Thr Val Ser Ile Lys Ser Leu
  65          70          75          80
Lys Phe Cys His Ser Gln Leu Ser Asn Asn Ser Val Ser Phe Phe Leu
          85          90          95
Tyr Asn Leu Asp His Ser His Ala Asn Tyr Tyr Phe Cys Asn Leu Ser
          100          105          110
Ile Phe Asp Pro Pro Pro Phe Lys Val Thr Leu Thr Gly Gly Tyr Leu
          115          120          125
His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Phe Trp Leu Pro
          130          135          140

```



Ile Gly Cys Ala Ala Phe Val Val Val Cys Ile Leu Gly Cys Ile Leu  
145 150 155 160  
Ile Cys Trp Leu Thr Lys Lys Lys Tyr Ser Ser Ser Val His Asp Pro  
165 170 175  
Asn Gly Glu Tyr Met Phe Met Arg Ala Val Asn Thr Ala Lys Lys Ser  
180 185 190  
Arg Leu Thr Asp Val Thr Leu  
195

<210> 24  
<211> 200  
<212> PRT  
<213> mouse

<400> 24  
Met Lys Pro Tyr Phe Cys Arg Val Phe Val Phe Cys Phe Leu Ile Arg  
1 5 10 15  
Leu Leu Thr Gly Glu Ile Asn Gly Ser Ala Asp His Arg Met Phe Ser  
20 25 30  
Phe His Asn Gly Gly Val Gln Ile Ser Cys Lys Tyr Pro Glu Thr Val  
35 40 45  
Gln Gln Leu Lys Met Arg Leu Phe Arg Glu Arg Glu Val Leu Cys Glu  
50 55 60  
Leu Thr Lys Thr Lys Gly Ser Gly Asn Ala Val Ser Ile Lys Asn Pro  
65 70 75 80  
Met Leu Cys Leu Tyr His Leu Ser Asn Asn Ser Val Ser Phe Phe Leu  
85 90 95  
Asn Asn Pro Asp Ser Ser Gln Gly Ser Tyr Tyr Phe Cys Ser Leu Ser  
100 105 110  
Ile Phe Asp Pro Pro Pro Phe Gln Glu Arg Asn Leu Ser Gly Gly Tyr  
115 120 125  
Leu His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Leu Trp Leu  
130 135 140  
Pro Val Gly Cys Ala Ala Phe Val Val Val Leu Leu Phe Gly Cys Ile  
145 150 155 160  
Leu Ile Ile Trp Phe Ser Lys Lys Lys Tyr Gly Ser Ser Val His Asp  
165 170 175  
Pro Asn Ser Glu Tyr Met Phe Met Ala Ala Val Asn Thr Asn Lys Lys  
180 185 190  
Ser Arg Leu Ala Gly Val Thr Ser  
195 200

<210> 25  
<211> 24  
<212> DNA  
<213> Artificial Sequence

003211 02482260

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 Oglionucleotide

<400> 25  
 accatgcggc tgggcagtcc tgga 24

<210> 26  
 <211> 23  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 Oglionucleotide

<400> 26  
 tggtgaccta ccacatccca cag 23

<210> 27  
 <211> 23  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 Oglionucleotide

<400> 27  
 tccgatgtca tttcctgtct ggc 23

<210> 28  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 Oglionucleotide

<400> 28  
 gctctgtctc cggactcaca gccc 24

<210> 29  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:Synthetic  
 Oglionucleotide

<400> 29  
 gtggcagcaa acttcagcgt gcccgtcg 28

<210> 30  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence

00341043460

<220>  
<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

<400> 30  
cccaacgtgt actggatcaa taagacgg 28

<210> 31  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

<400> 31  
gcgtgctgag gatcgacagg acccccag 28

<210> 32  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

<400> 32  
gcctctagaa agagctggga c 21

<210> 33  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

<400> 33  
cgccgtgttc catttatgag c 21

<210> 34  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

<400> 34  
gcatatttat gaatccca 18

<210> 35  
<211> 18  
<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic  
Oglionucleotide

<400> 35

actattaggg tcatgcac

18

00227 02432.60